OLIGODEOXYNUCLEOTIDE Xol26

MstII

5'-CCCGGGAAGCTT$\boxed{\text{CCTTAGG}}$CTTAAAGAAAGTGGTGCTGGGCAAAAAAGGG-3'

prepro-HSA ← → V1 domain of CD4 receptor

OLIGODEOXYNUCLEOTIDE Xol27

SmaI HindIII

5'-<u>CCCGGG</u>$\boxed{\text{AAGCTT}}$TTAGAAAGCTAGCACCACGATGTCTAT-3'

→ V2 domain of CD4 receptor

stop codon

Figure 1

```
 MstII
 CCTTAGGCTTAAAGAAAGTGGTGCTGGGCAAAAAAGGGGATACAGTGGAACTGACCTGTACAGCTTCCCAGAAGA
01            11            21            31            41            51            61            71

 AGAGCATACAATTCCACTGGAAAAACTCCAACCAGATAAAGATTCTGGGAAATCAGGGCTCCTTCTTAACTAAAG
76            86            96            106           116           126           136           146

 GTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAAGAAGCCTTTGGGACCAAGGAAACTTCCCCCTGATCATCA
151           161           171           181           191           201           211           221

 AGAATCTTAAGATAGAAGACTCAGATACTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTGCTAG
226           236           246           256           266           276           286           296

 TGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACCCTGACCTTGGAGAGCCCCC
301           311           321           331           341           351           361           371

 CTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGTAAAAACATACAGGGGGGGAAGACCCTCTCCGTGT
376           386           396           406           416           426           436           446

 CTCAGCTGGAGCTCCAGGATAGTGGCACCTGGACATGCACTGTCTTGCAGAACCAGAAGAAGGTGGAGTTCAAAA
451           461           471           481           491           501           511           521

                                        HindIII  SmaI
 TAGACATCGTGGTGCTAGCTTTCTAAAAGCTTCCCGGG
526           536           546           556
```

Figure 2

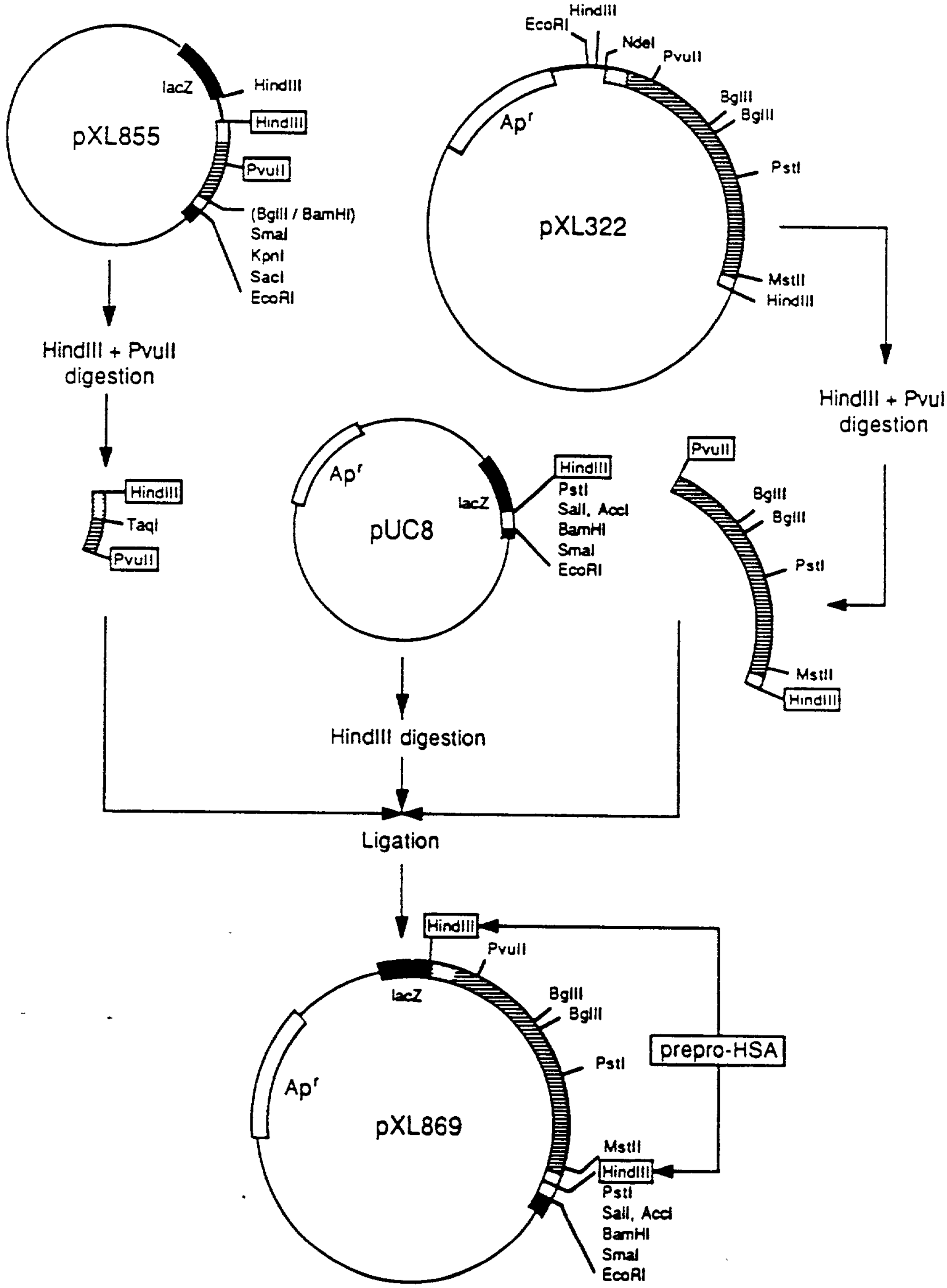
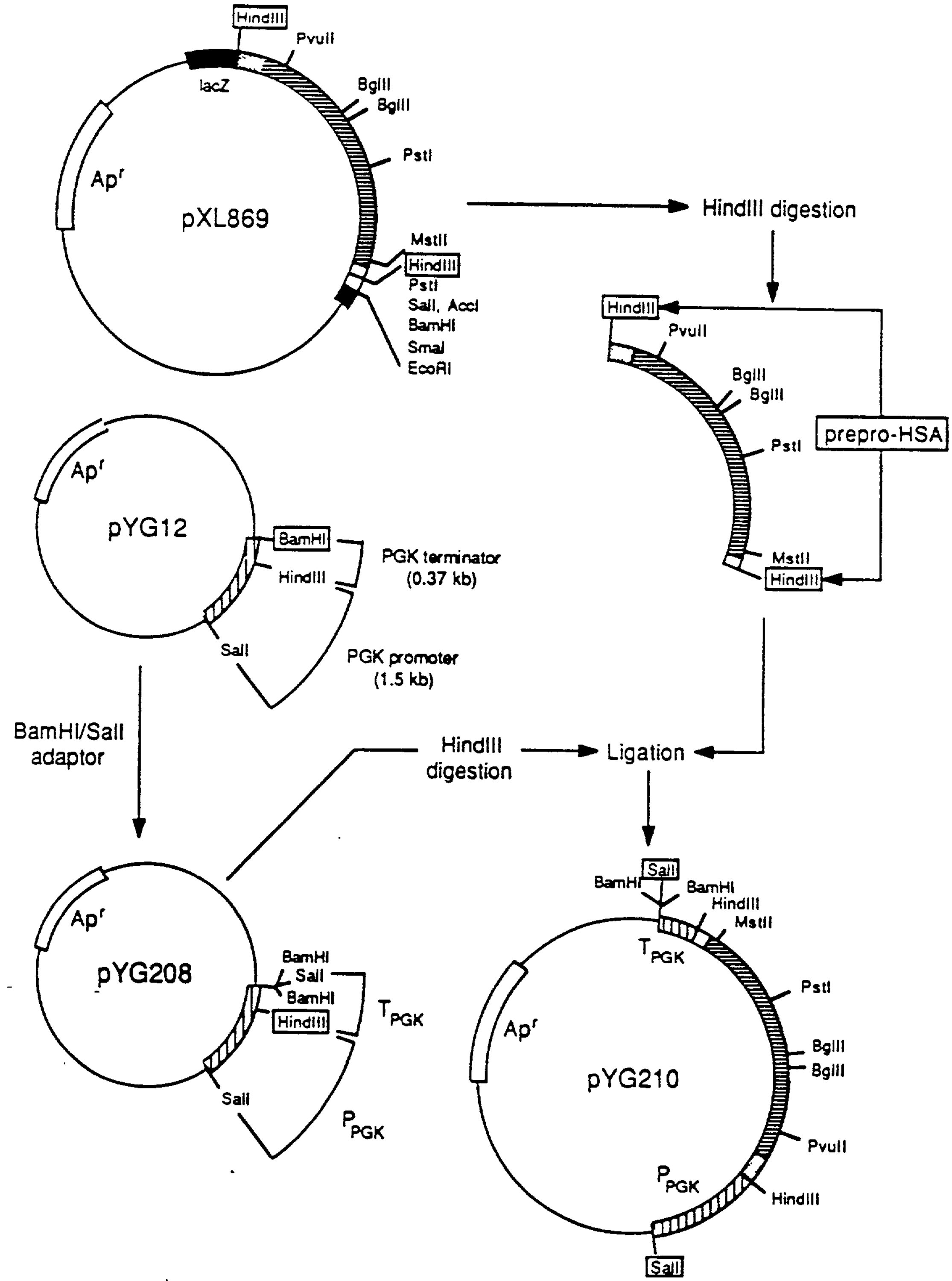

pXL855
lacZ
HindIII
HindIII
PvuII
(BglII / BamHI)
SmaI
KpnI
SacI
EcoRI
HindIII + PvuII
digestion
HindIII
TaqI
PvuII
pXL322
EcoRI
HindIII
NdeI
PvuII
Ap^r
BglII
BglII
PstI
MstII
HindIII
HindIII + PvuII
digestion
PvuII
BglII
BglII
PstI
MstII
HindIII
pUC8
Ap^r
lacZ
HindIII
PstI
SalI, AccI
BamHI
SmaI
EcoRI
HindIII digestion
Ligation
pXL869
HindIII
PvuII
lacZ
BglII
BglII
PstI
Ap^r
prepro-HSA
MstII
HindIII
PstI
SalI, AccI
BamHI
SmaI
EcoRI

Figure 3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PvuII
lacZ
BglII
BglII
PstI
Ap^r
pXL869
MstII
HindIII
PstI
SalI, AccI
BamHI
SmaI
EcoRI
HindIII digestion
HindIII
PvuII
BglII
BglII
PstI
prepro-HSA
MstII
HindIII
Ap^r
pYG12
BamHI
HindIII
PGK terminator
(0.37 kb)
SalI
PGK promoter
(1.5 kb)
BamHI/SalI
adaptor
HindIII
digestion
Ligation
Ap^r
pYG208
BamHI
SalI
BamHI
HindIII
T_PGK
SalI
P_PGK
SalI
BamHI
BamHI
HindIII
MstII
T_PGK
PstI
Ap^r
BglII
BglII
pYG210
PvuII
P_PGK
HindIII
SalI

Figure 4

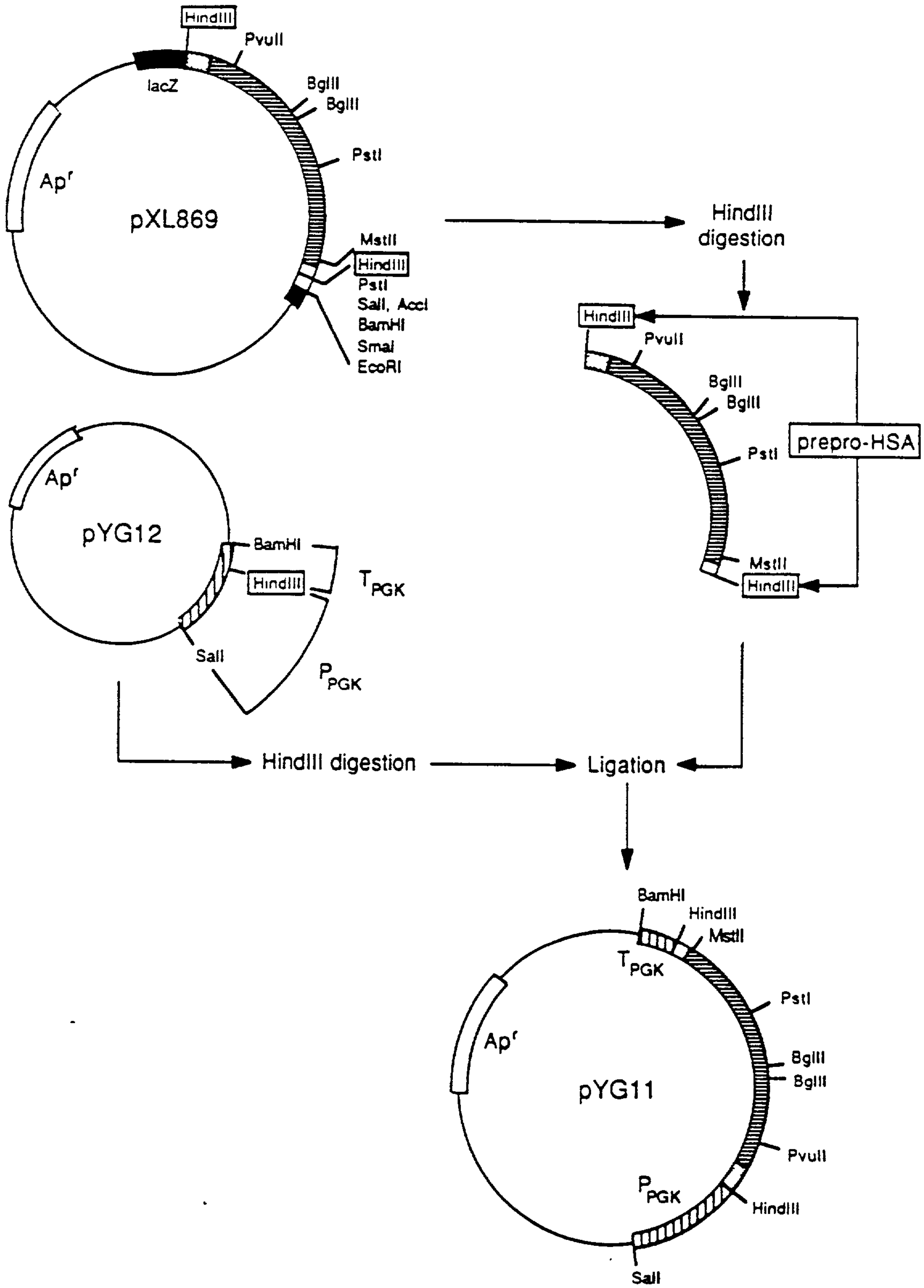

HindIII
PvuII
lacZ
BglII
BglII
PstI
Apr
pXL869
MstII
HindIII
PstI
SalI, AccI
BamHI
SmaI
EcoRI
HindIII
digestion
HindIII
PvuII
BglII
BglII
PstI
prepro-HSA
MstII
HindIII
Apr
pYG12
BamHI
HindIII
TPGK
SalI
PPGK
HindIII digestion
Ligation
BamHI
HindIII
MstII
TPGK
PstI
Apr
BglII
BglII
pYG11
PvuII
PPGK
HindIII
SalI

Figure 5

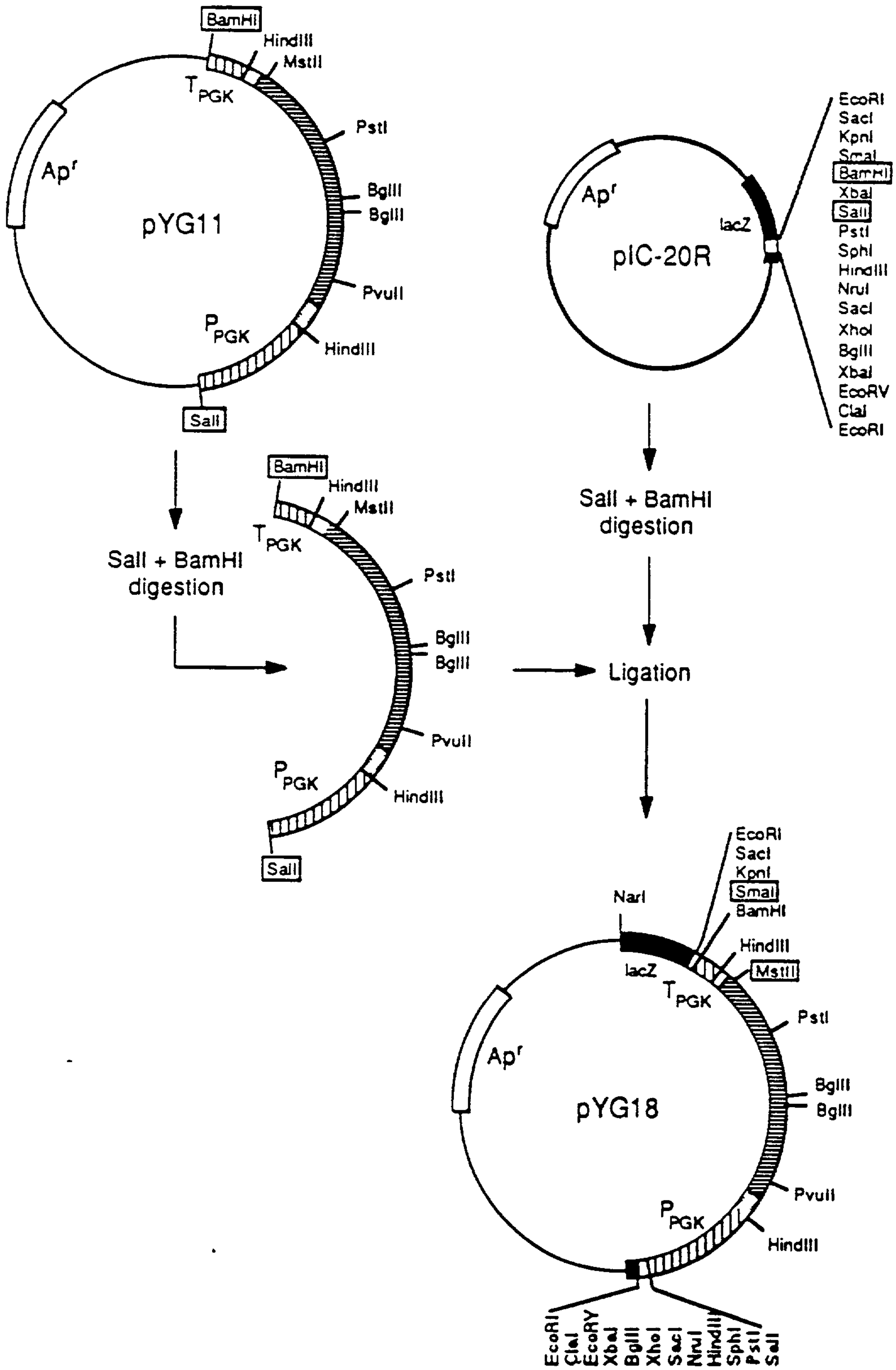
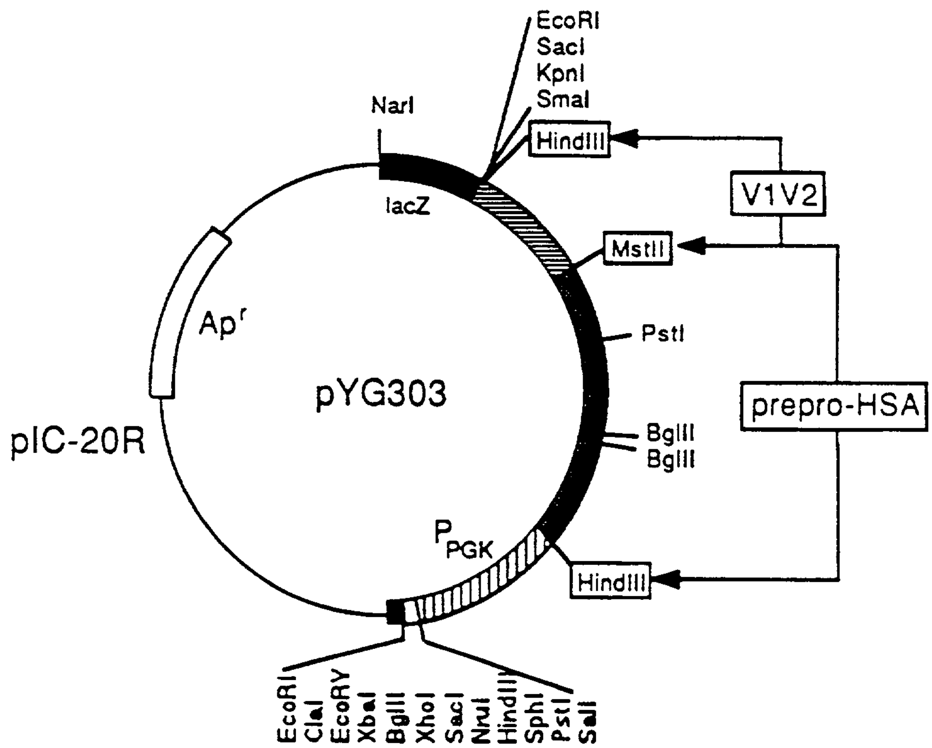

BamHI
HindIII
MstII
T PGK
PstI
BglII
BglII
PvuII
P PGK
HindIII
SalI
Ap r
pYG11
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
HindIII
NruI
SacI
XhoI
BglII
XbaI
EcoRV
ClaI
EcoRI
Ap r
lacZ
pIC-20R
SalI + BamHI
digestion
SalI + BamHI
digestion
Ligation
NarI
lacZ
EcoRI
SacI
KpnI
SmaI
BamHI
HindIII
MstII
pYG18
Ap r
EcoRI
ClaI
EcoRV
XbaI
BglII
XhoI
SacI
NruI
HindIII
SphI
PstI
SalI

Figure 6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SacI
KpnI
SmaI
NarI
HindIII
lacZ
V1V2
MstII
Ap^r
PstI
pYG303
prepro-HSA
BglII
BglII
pIC-20R
P PGK
HindIII
EcoRI
ClaI
EcoRV
XbaI
BglII
XhoI
SacI
NruI
HindIII
SphI
PstI
SalI

Figure 7

```
      MetLysTrpValThrPheIleSerLeuLeuPheLeuPheSerSerAlaTyrSerArgGlyValPheArg
 AAGCTTATGAAGTGGGTAACCTTTATTTCCCTTCTTTTTCTCTTTAGCTCGGCTTATTCCAGGGGTGTGTTTCGT
 1         11        21        31        41        51        61        71

 ArgAspAlaHisLysSerGluValAlaHisArgPheLysAspLeuGlyGluGluAsnPheLysAlaLeuValLeu
 CGAGATGCACACAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGGGAGAAGAAAATTTCAAAGCCTTGGTGTTG
76         86        96        106       116       126       136       146

 IleAlaPheAlaGlnTyrLeuGlnGlnCysProPheGluAspHisValLysLeuValAsnGluValThrGluPhe
 ATTGCCTTTGCTCAGTATCTTCAGCAGTGTCCATTTGAAGATCATGTAAAATTAGTGAATGAAGTAACTGAATTT
151        161       171       181       191       201       211       221

 AlaLysThrCysValAlaAspGluSerAlaGluAsnCysAspLysSerLeuHisThrLeuPheGlyAspLysLeu
 GCAAAAACATGTGTTGCTGATGAGTCAGCTGAAAATTGTGACAAATCACTTCATACCCTTTTTGGAGACAAATTA
226        236       246       256       266       276       286       296

 CysThrValAlaThrLeuArgGluThrTyrGlyGluMetAlaAspCysCysAlaLysGlnGluProGluArgAsn
 TGCACAGTTGCAACTCTTCGTGAAACCTATGGTGAAATGGCTGACTGCTGTGCAAAACAAGAACCTGAGAGAAAT
301        311       321       331       341       351       361       371

 GluCysPheLeuGlnHisLysAspAspAsnProAsnLeuProArgLeuValArgProGluValAspValMetCys
 GAATGCTTCTTGCAACACAAAGATGACAACCCAAACCTCCCCCGATTGGTGAGACCAGAGGTTGATGTGATGTGC
376        386       396       406       416       426       436       446

 ThrAlaPheHisAspAsnGluGluThrPheLeuLysLysTyrLeuTyrGluIleAlaArgArgHisProTyrPhe
 ACTGCTTTTCATGACAATGAAGAGACATTTTTGAAAAAATACTTATATGAAATTGCCAGAAGACATCCTTACTTT
451        461       471       481       491       501       511       521

 TyrAlaProGluLeuLeuPhePheAlaLysArgTyrLysAlaAlaPheThrGluCysCysGlnAlaAlaAspLys
 TATGCCCCGGAACTCCTTTTCTTTGCTAAAAGGTATAAAGCTGCTTTTACAGAATGTTGCCAAGCTGCTGATAAA
526        536       546       556       566       576       586       596

 AlaAlaCysLeuLeuProLysLeuAspGluLeuArgAspGluGlyLysAlaSerSerAlaLysGlnArgLeuLys
 GCTGCCTGCCTGTTGCCAAAGCTCGATGAACTTCGGGATGAAGGGAAGGCTTCGTCTGCCAAACAGAGACTCAAG
601        611       621       631       641       651       661       671

 CysAlaSerLeuGlnLysPheGlyGluArgAlaPheLysAlaTrpAlaValAlaArgLeuSerGlnArgPhePro
 TGTGCCAGTCTCCAAAAATTTGGAGAAAGAGCTTTCAAAGCATGGGCAGTAGCTCGCCTGAGCCAGAGATTTCCC
676        686       696       706       716       726       736       746

 LysAlaGluPheAlaGluValSerLysLeuValThrAspLeuThrLysValHisThrGluCysCysHisGlyAsp
 AAAGCTGAGTTTGCAGAAGTTTCCAAGTTAGTGACAGATCTTACCAAAGTCCACACGGAATGCTGCCATGGAGAT
751        761       771       781       791       801       811       821

 LeuLeuGluCysAlaAspAspArgAlaAspLeuAlaLysTyrIleCysGluAsnGlnAspSerIleSerSerLys
 CTGCTTGAATGTGCTGATGACAGGGCGGACCTTGCCAAGTATATCTGTGAAAATCAAGATTCGATCTCCAGTAAA
826        836       846       856       866       876       886       896

 LeuLysGluCysCysGluLysProLeuLeuGluLysSerHisCysIleAlaGluValGluAsnAspGluMetPro
 CTGAAGGAATGCTGTGAAAAACCTCTGTTGGAAAAATCCCACTGCATTGCCGAAGTGGAAAATGATGAGATGCCT
901        911       921       931       941       951       961       971

 AlaAspLeuProSerLeuAlaAlaAspPheValGluSerLysAspValCysLysAsnTyrAlaGluAlaLysAsp
 GCTGACTTGCCTTCATTAGCTGCTGATTTTGTTGAAAGTAAGGATGTTTGCAAAAACTATGCTGAGGCAAAGGAT
976        986       996       1006      1016      1026      1036      1046
```

Figure 8A

```
     ValPheLeuGlyMetPheLeuTyrGluTyrAlaArgArgHisProAspTyrSerValValLeuLeuLeuArgLeu
     GTCTTCCTGGGCATGTTTTTGTATGAATATGCAAGAAGGCATCCTGATTACTCTGTCGTACTGCTGCTGAGACTT
1051      1061      1071      1081      1091      1101      1111      1121

     AlaLysThrTyrGluThrThrLeuGluLysCysCysAlaAlaAlaAspProHisGluCysTyrAlaLysValPhe
     GCCAAGACATATGAAACCACTCTAGAGAAGTGCTGTGCCGCTGCAGATCCTCATGAATGCTATGCCAAAGTGTTC
1126      1136      1146      1156      1166      1176      1186      1196

     AspGluPheLysProLeuValGluGluProGlnAsnLeuIleLysGlnAsnCysGluLeuPheGluGlnLeuGly
     GATGAATTTAAACCTCTTGTGGAAGAGCCTCAGAATTTAATCAAACAAAATTGTGAGCTTTTTGAGCAGCTTGGA
1201      1211      1221      1231      1241      1251      1261      1271

     GluTyrLysPheGlnAsnAlaLeuLeuValArgTyrThrLysLysValProGlnValSerThrProThrLeuVal
     GAGTACAAATTCCAGAATGCGCTATTAGTTCGTTACACCAAGAAAGTACCCCAAGTGTCAACTCCAACTCTTGTA
1276      1286      1296      1306      1316      1326      1336      1346

     GluValSerArgAsnLeuGlyLysValGlySerLysCysCysLysHisProGluAlaLysArgMetProCysAla
     GAGGTCTCAAGAAACCTAGGAAAAGTGGGCAGCAAATGTTGTAAACATCCTGAAGCAAAAAGAATGCCCTGTGCA
1351      1361      1371      1381      1391      1401      1411      1421

     GluAspTyrLeuSerValValLeuAsnGlnLeuCysValLeuHisGluLysThrProValSerAspArgValThr
     GAAGACTATCTATCCGTGGTCCTGAACCAGTTATGTGTGTTGCATGAGAAAACGCCAGTAAGTGACAGAGTCACC
1426      1436      1446      1456      1466      1476      1486      1496

     LysCysCysThrGluSerLeuValAsnArgArgProCysPheSerAlaLeuGluValAspGluThrTyrValPro
     AAATGCTGCACAGAATCCTTGGTGAACAGGCGACCATGCTTTTCAGCTCTGGAAGTCGATGAAACATACGTTCCC
1501      1511      1521      1531      1541      1551      1561      1571

     LysGluPheAsnAlaGluThrPheThrPheHisAlaAspIleCysThrLeuSerGluLysGluArgGlnIleLys
     AAAGAGTTTAATGCTGAAACATTCACCTTCCATGCAGATATATGCACACTTTCTGAGAAGGAGAGACAAATCAAG
1576      1586      1596      1606      1616      1626      1636      1646

     LysGlnThrAlaLeuValGluLeuValLysHisLysProLysAlaThrLysGluGlnLeuLysAlaValMetAsp
     AAACAAACTGCACTTGTTGAGCTTGTGAAACACAAGCCCAAGGCAACAAAAGAGCAACTGAAAGCTGTTATGGAT
1651      1661      1671      1681      1691      1701      1711      1721

     AspPheAlaAlaPheValGluLysCysCysLysAlaAspAspLysGluThrCysPheAlaGluGluGlyLysLys
     GATTTCGCAGCTTTTGTAGAGAAGTGCTGCAAGGCTGACGATAAGGAGACCTGCTTTGCCGAGGAGGGTAAAAAA
1726      1736      1746      1756      1766      1776      1786      1796

     LeuValAlaAlaSerGlnAlaAlaLeuGlyLeuLysLysValValLeuGlyLysLysGlyAspThrValGluLeu
     CTTGTTGCTGCAAGTCAAGCTGCCTTAGGCTTAAAGAAAGTGGTGCTGGGCAAAAAGGGGATACAGTGGAACTG
1801      1811      1821      1831      1841      1851      1861      1871

     ThrCysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGlnIleLysIleLeuGlyAsn
     ACCTGTACAGCTTCCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGATAAAGATTCTGGGAAAT
1876      1886      1896      1906      1916      1926      1936      1946

     GlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArgAlaAspSerArgArgSerLeuTrpAspGln
     CAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAAGAAGCCTTTGGGACCAA
1951      1961      1971      1981      1991      2001      2011      2021

     GlyAsnPheProLeuIleIleLysAsnLeuLysIleGluAspSerAspThrTyrIleCysGluValGluAspGln
     GGAAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACTCAGATACTTACATCTGTGAAGTGGAGGACCAG
2026      2036      2046      2056      2066      2076      2086      2096

     LysGluGluValGlnLeuLeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeuGlnGlyGlnSerLeu
     AAGGAGGAGGTGCAATTGCTAGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTG
2101      2111      2121      2131      2141      2151      2161      2171
```

Figure 8B

```
   ThrLeuThrLeuGluSerProProGlySerSerProSerValGlnCysArgSerProArgGlyLysAsnIleGln
   ACCCTGACCTTGGAGAGCCCCCCTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGTAAAAACATACAG
2176      2186      2196      2206      2216      2226      2236      2246

   GlyGlyLysThrLeuSerValSerGlnLeuGluLeuGlnAspSerGlyThrTrpThrCysThrValLeuGlnAsn
   GGGGGGAAGACCCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGCACCTGGACATGCACTGTCTTGCAGAAC
2251      2261      2271      2281      2291      2301      2311      2321

   GlnLysLysValGluPheLysIleAspIleValValLeuAlaPhe***
   CAGAAGAAGGTGGAGTTCAAAATAGACATCGTGGTGCTAGCTTTCTAAAAGCTT
2326      2336      2346      2356      2366      2376
```

Figure 8C

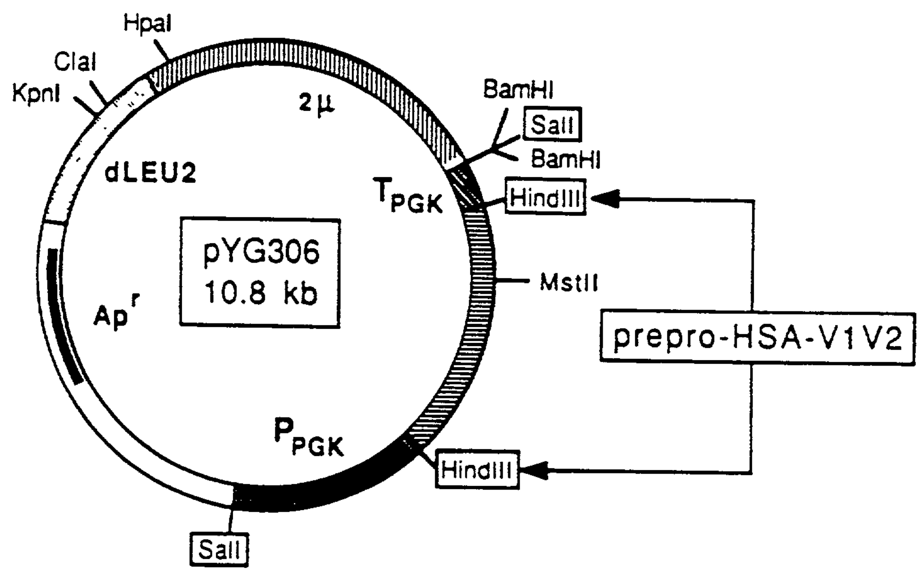

HpaI
ClaI
KpnI
2μ
dLEU2
BamHI
SalI
BamHI
T_{PGK}
HindIII
pYG306
10.8 kb
MstII
Ap^r
prepro-HSA-V1V2
P_{PGK}
HindIII
SalI

Figure 9

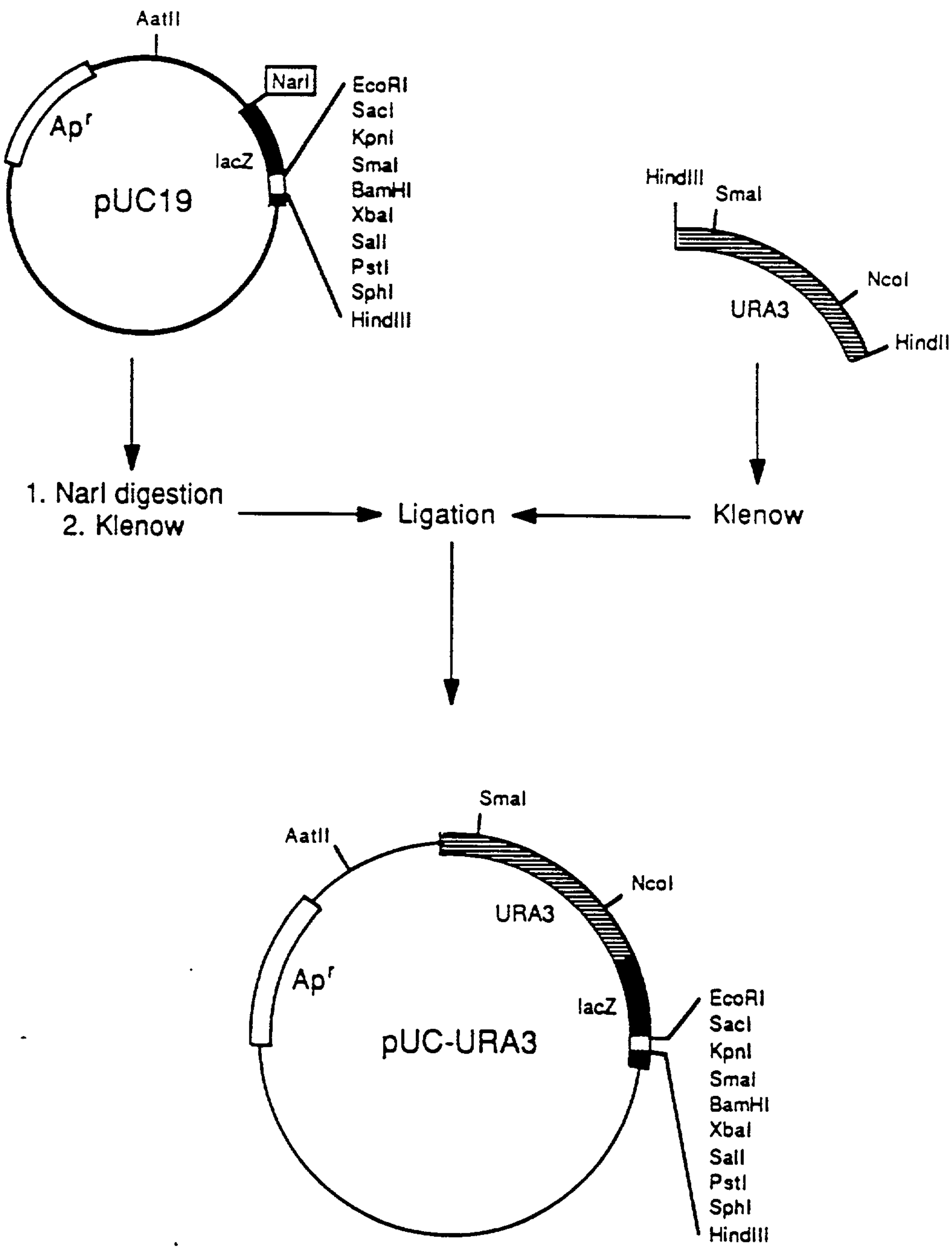

AatII
NarI
Ap^r
lacZ
pUC19
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
HindIII
HindIII
SmaI
NcoI
URA3
HindIII
1. NarI digestion
2. Klenow
Ligation
Klenow
SmaI
AatII
NcoI
URA3
Ap^r
lacZ
pUC-URA3
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
HindIII

Figure 10

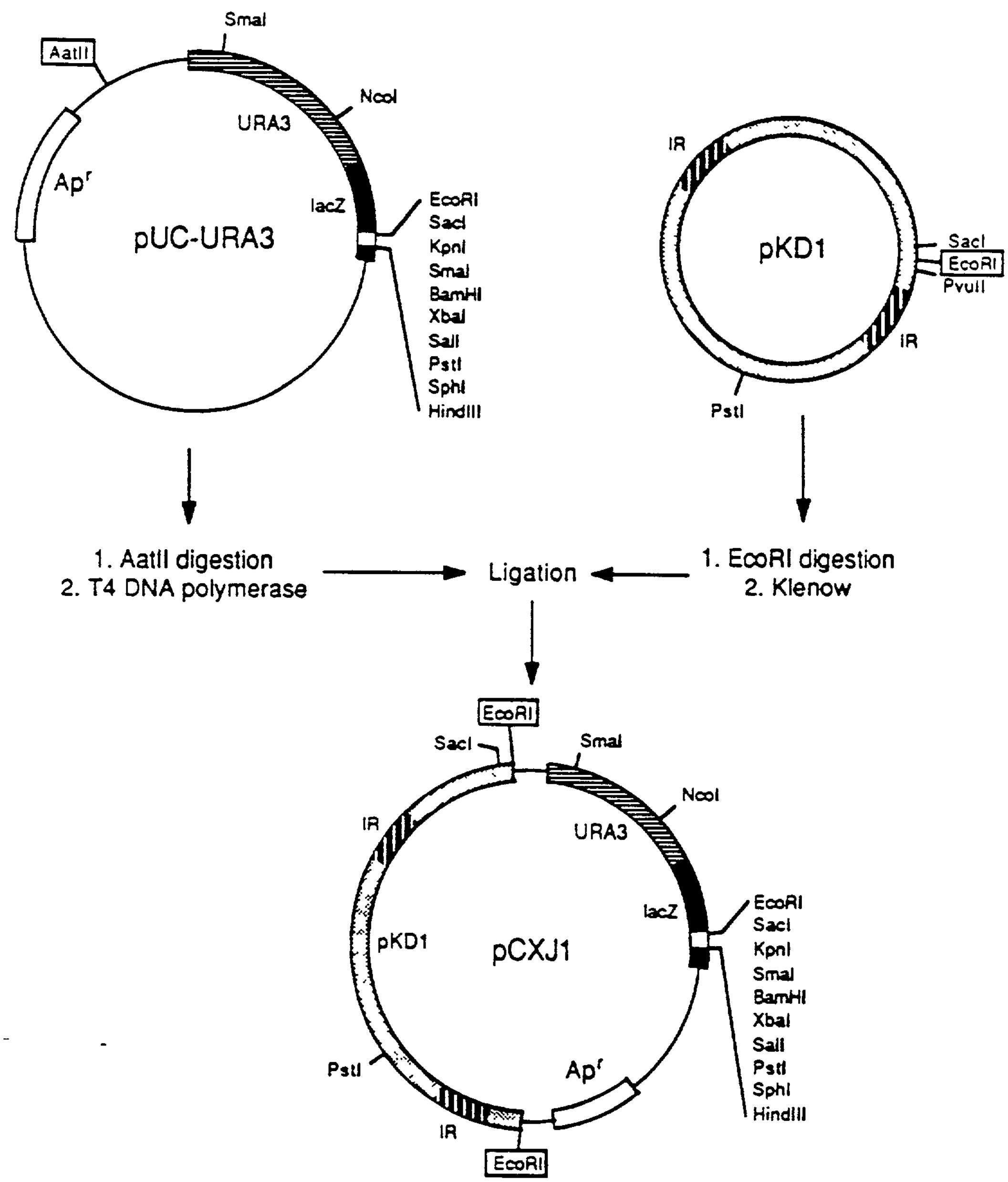
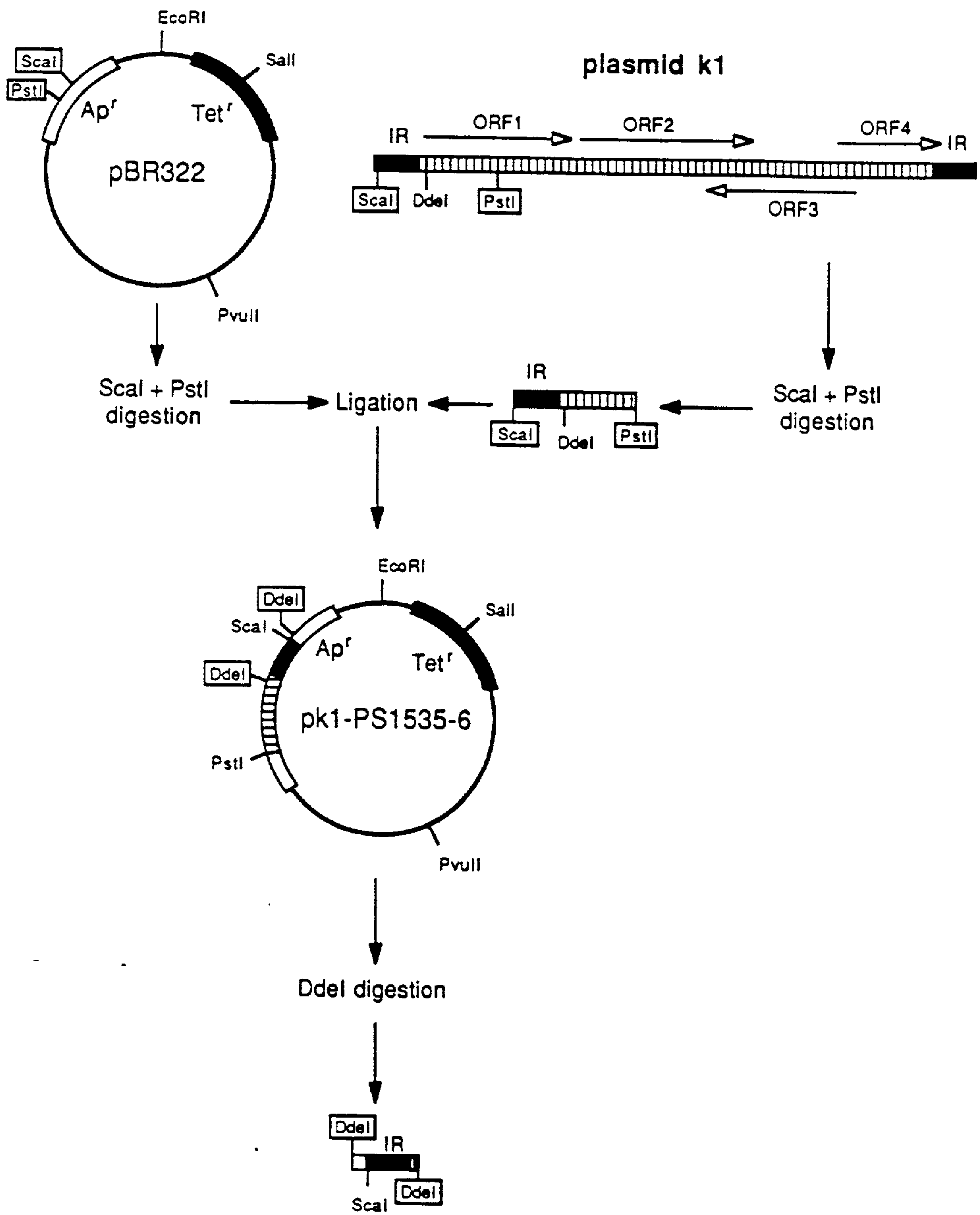

SmaI
AatII
NcoI
URA3
Ap^r
lacZ
pUC-URA3
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
HindIII
IR
pKD1
SacI
EcoRI
PvuII
IR
PstI
1. AatII digestion
2. T4 DNA polymerase
Ligation
1. EcoRI digestion
2. Klenow
EcoRI
SacI
SmaI
NcoI
IR
URA3
lacZ
pKD1
pCXJ1
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
HindIII
PstI
Ap^r
IR
EcoRI

Figure 11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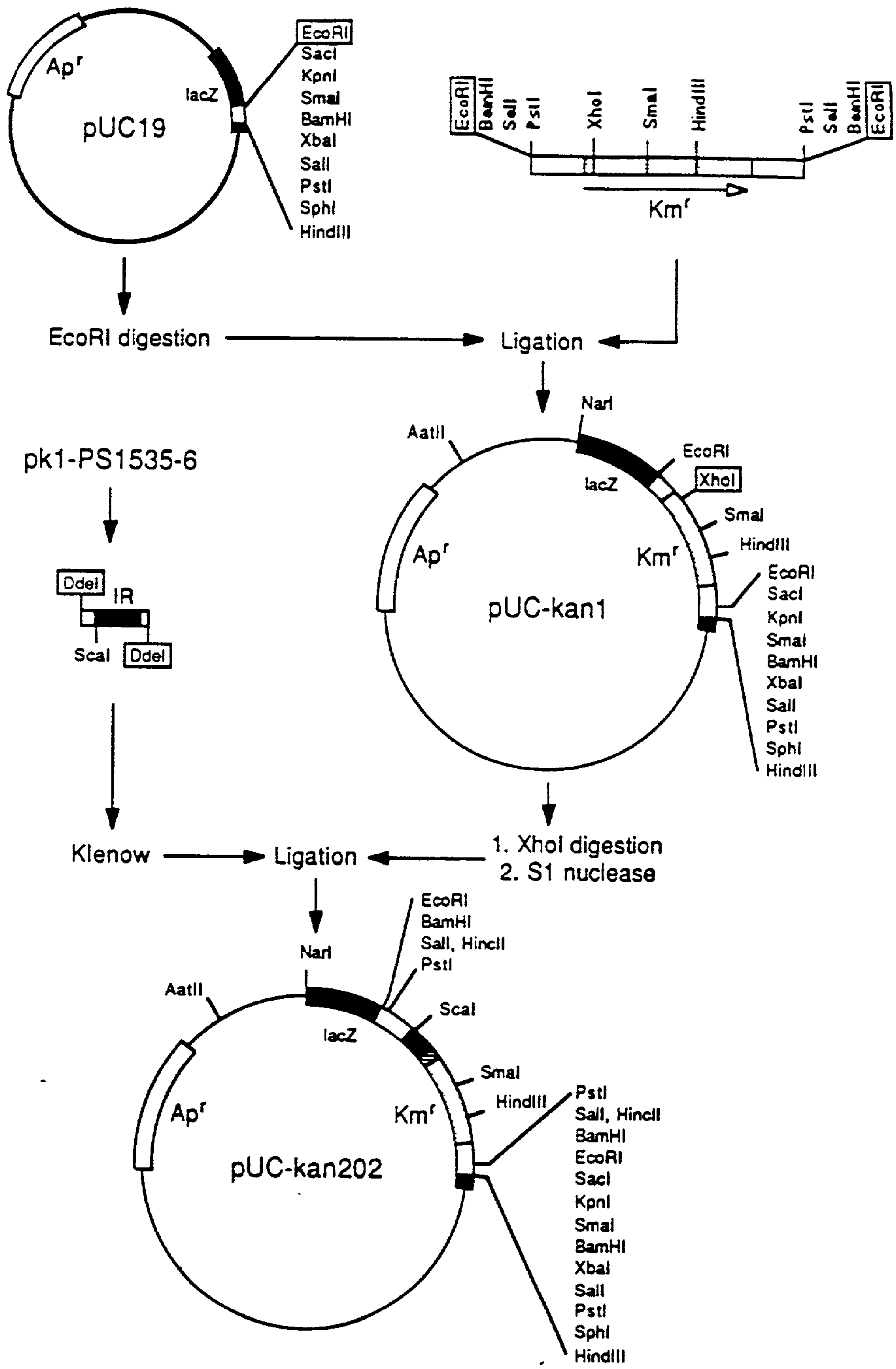
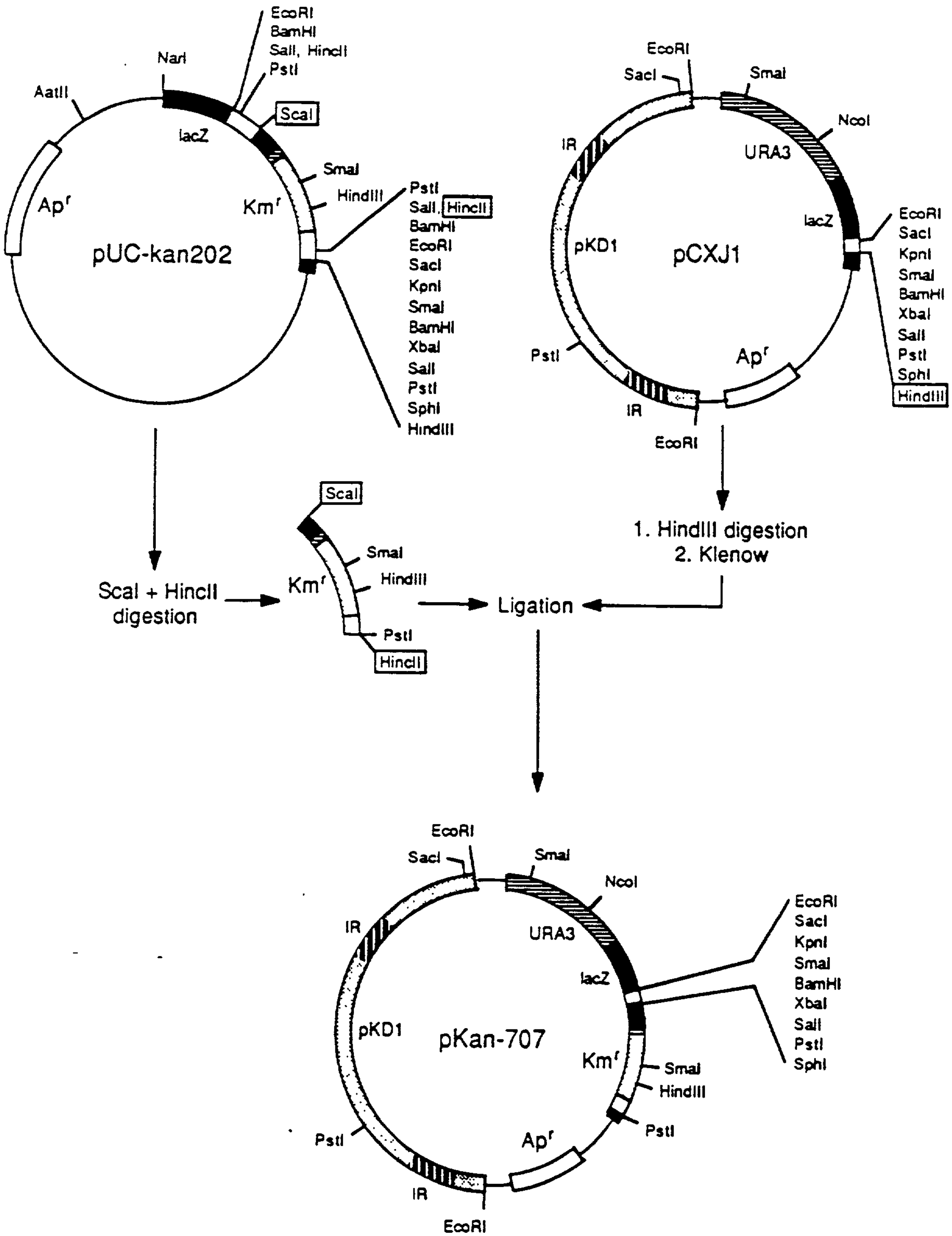
ScaI
PstI
SalI
Ap^r
Tet^r
pBR322
PvuII
plasmid k1
IR
ORF1
ORF2
ORF4
IR
ScaI
DdeI
PstI
ORF3
ScaI + PstI
digestion
Ligation
IR
ScaI
DdeI
PstI
ScaI + PstI
digestion
EcoRI
DdeI
SalI
ScaI
Ap^r
Tet^r
DdeI
pk1-PS1535-6
PstI
PvuII
DdeI digestion
DdeI
IR
ScaI
DdeI

Figure 12



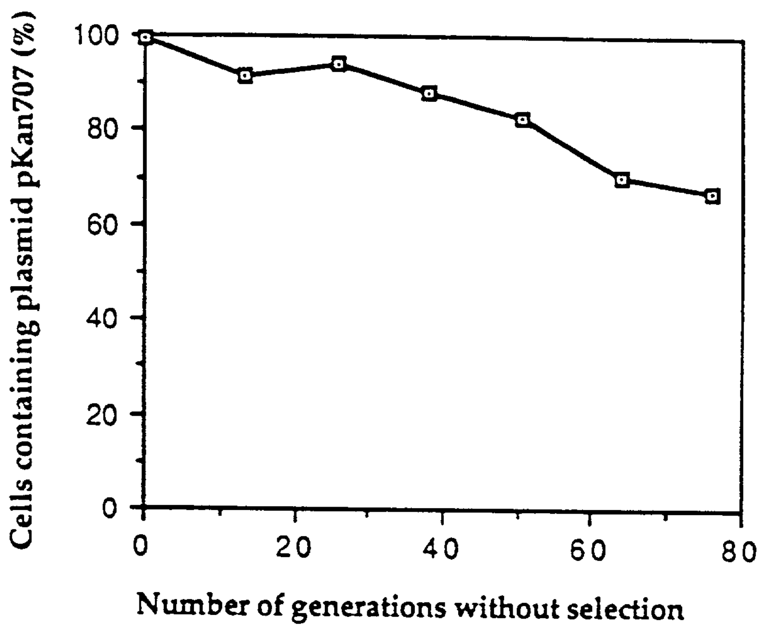
pUC19
Ap^r
lacZ
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
HindIII
EcoRI BamHI SalI PstI XhoI SmaI HindIII PstI SalI BamHI EcoRI
Km^r
EcoRI digestion
Ligation
NarI
AatII
EcoRI
XhoI
lacZ
SmaI
HindIII
Ap^r
Km^r
pUC-kan1
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
HindIII
pk1-PS1535-6
DdeI
IR
ScaI
DdeI
Klenow
Ligation
1. XhoI digestion
2. S1 nuclease
EcoRI
BamHI
SalI, HincII
PstI
NarI
AatII
ScaI
lacZ
SmaI
HindIII
Ap^r
Km^r
pUC-kan202
PstI
SalI, HincII
BamHI
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
HindIII

Figure 13



pUC-kan202
EcoRI
BamHI
SalI, HincII
PstI
NarI
AatII
ScaI
lacZ
SmaI
HindIII
Apr
Kmr
PstI
SalI, HincII
BamHI
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
HindIII
pCXJ1
EcoRI
SacI
SmaI
NcoI
IR
URA3
lacZ
pKD1
PstI
Apr
IR
EcoRI
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
HindIII
ScaI + HincII digestion
ScaI
SmaI
HindIII
Kmr
PstI
HincII
1. HindIII digestion
2. Klenow
Ligation
pKan-707
EcoRI
SacI
SmaI
NcoI
IR
URA3
lacZ
pKD1
Kmr
SmaI
HindIII
PstI
PstI
Apr
IR
EcoRI
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI

Figure 14


Cells containing plasmid pKan707 (%)
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80
60
40
20
0
0
20
40
60
80
Number of generations without selection

Figure 15

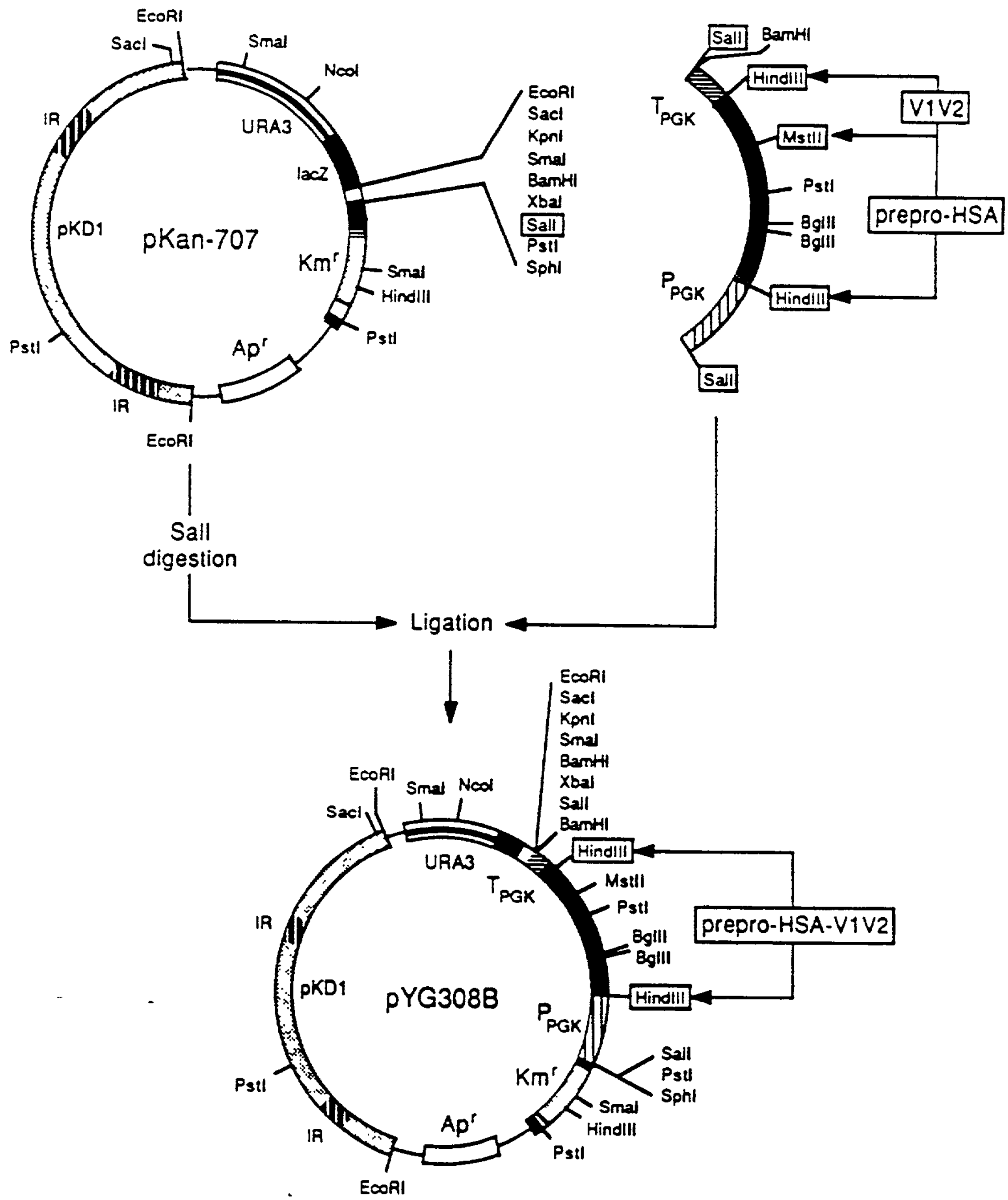
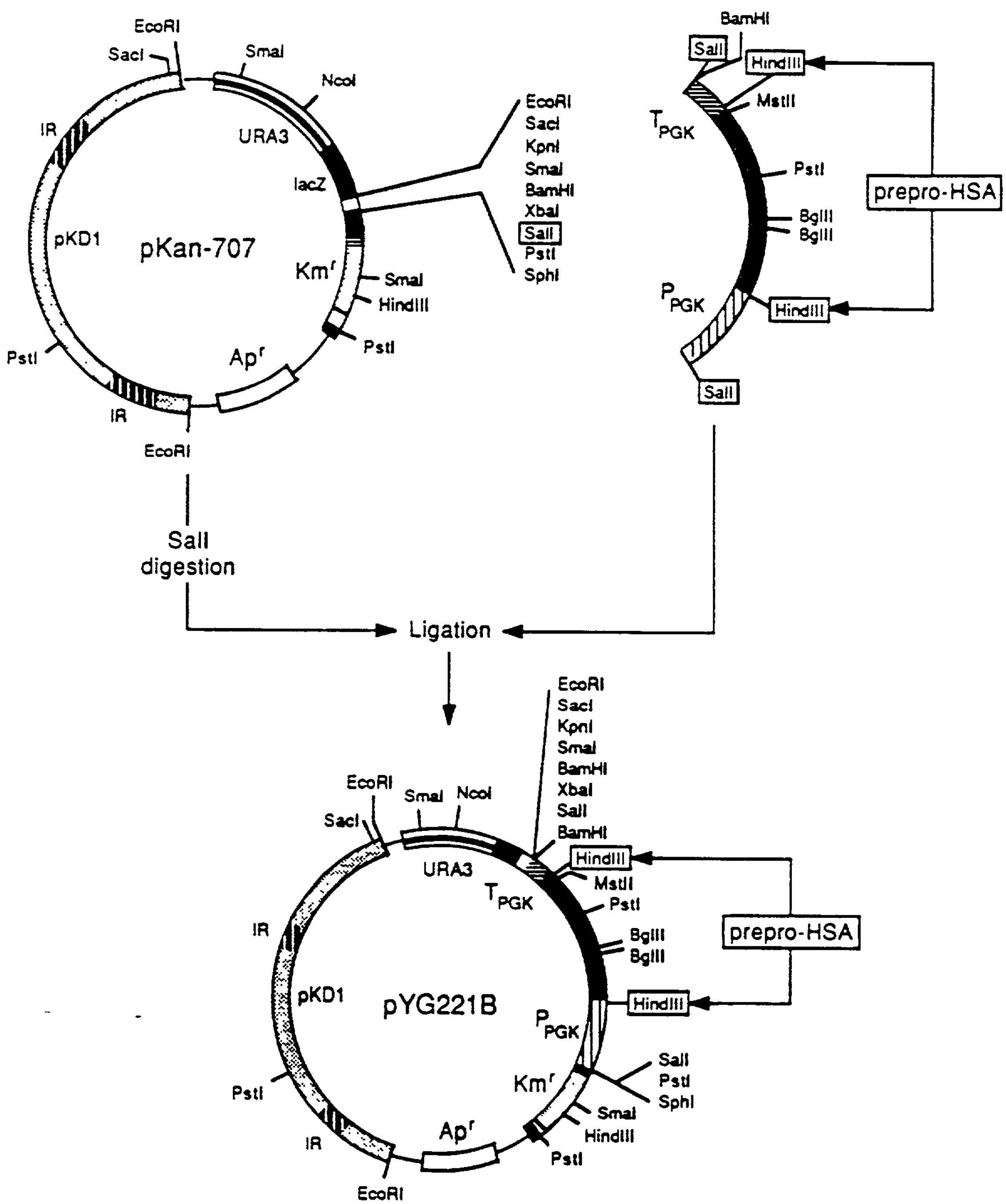

EcoRI
SacI
SmaI
NcoI
URA3
IR
lacZ
pKD1
pKan-707
Km^r
SmaI
HindIII
PstI
Ap^r
PstI
IR
EcoRI
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
SalI
BamHI
HindIII
V1V2
T$_{PGK}$
MstII
PstI
prepro-HSA
BglII
BglII
P$_{PGK}$
HindIII
SalI
SalI
digestion
Ligation
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
BamHI
EcoRI
SmaI
NcoI
SacI
HindIII
URA3
T$_{PGK}$
MstII
PstI
prepro-HSA-V1V2
IR
BglII
BglII
pKD1
pYG308B
HindIII
P$_{PGK}$
SalI
PstI
SphI
Km^r
PstI
SmaI
HindIII
IR
Ap^r
PstI
EcoRI

Figure 16



EcoRI
SacI
SmaI
NcoI
IR
URA3
lacZ
pKD1
pKan-707
Km^r
SmaI
HindIII
PstI
Ap^r
PstI
IR
EcoRI
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
SphI
BamHI
SalI
HindIII
MstII
T$_{PGK}$
PstI
BglII
BglII
prepro-HSA
P$_{PGK}$
HindIII
SalI
SalI
digestion
Ligation
EcoRI
SacI
KpnI
SmaI
BamHI
XbaI
SalI
BamHI
HindIII
MstII
PstI
BglII
BglII
prepro-HSA
HindIII
EcoRI
SmaI
NcoI
SacI
URA3
T$_{PGK}$
IR
pKD1
pYG221B
P$_{PGK}$
Km^r
SalI
PstI
SphI
SmaI
HindIII
PstI
PstI
Ap^r
IR
EcoRI

Figure 17

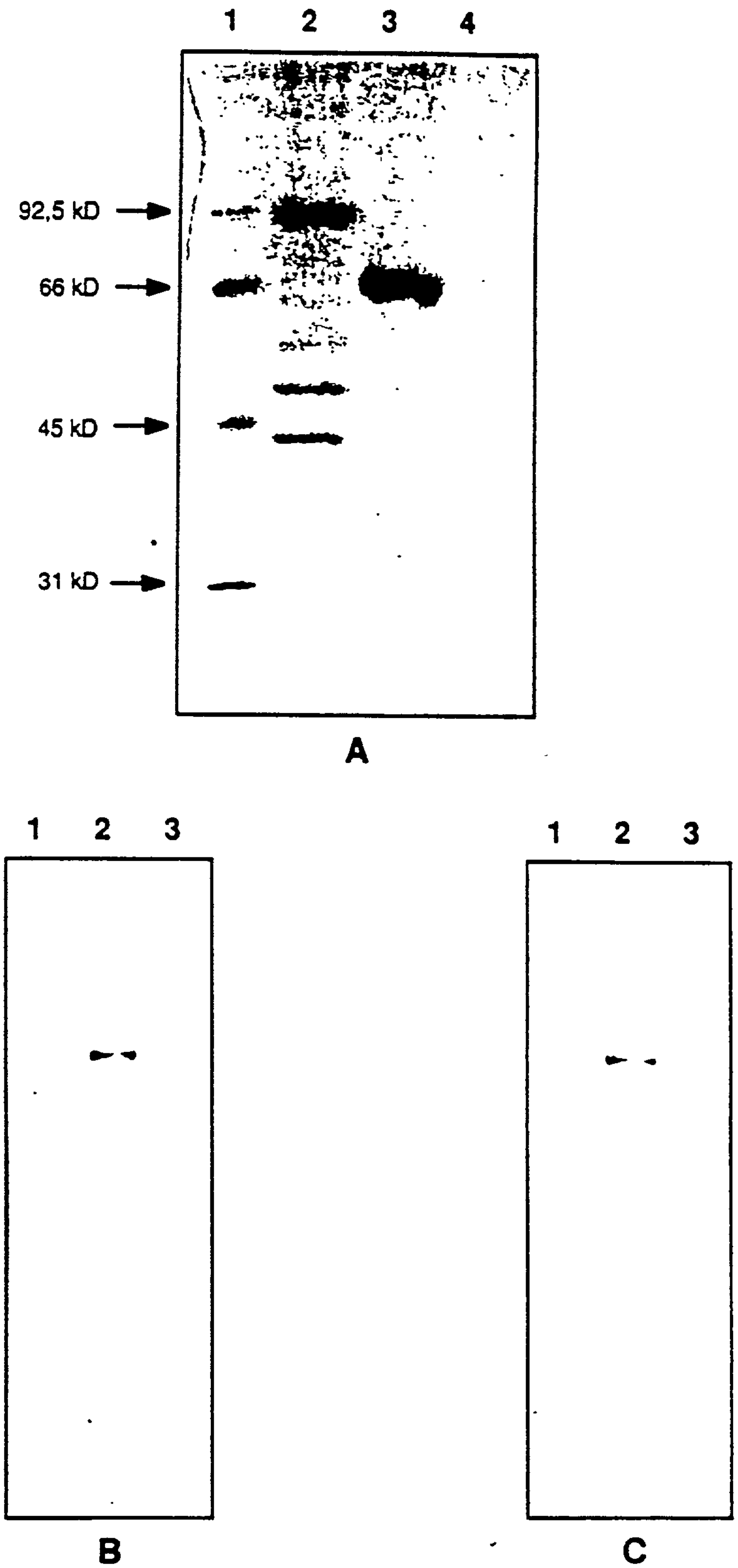


Figure 18

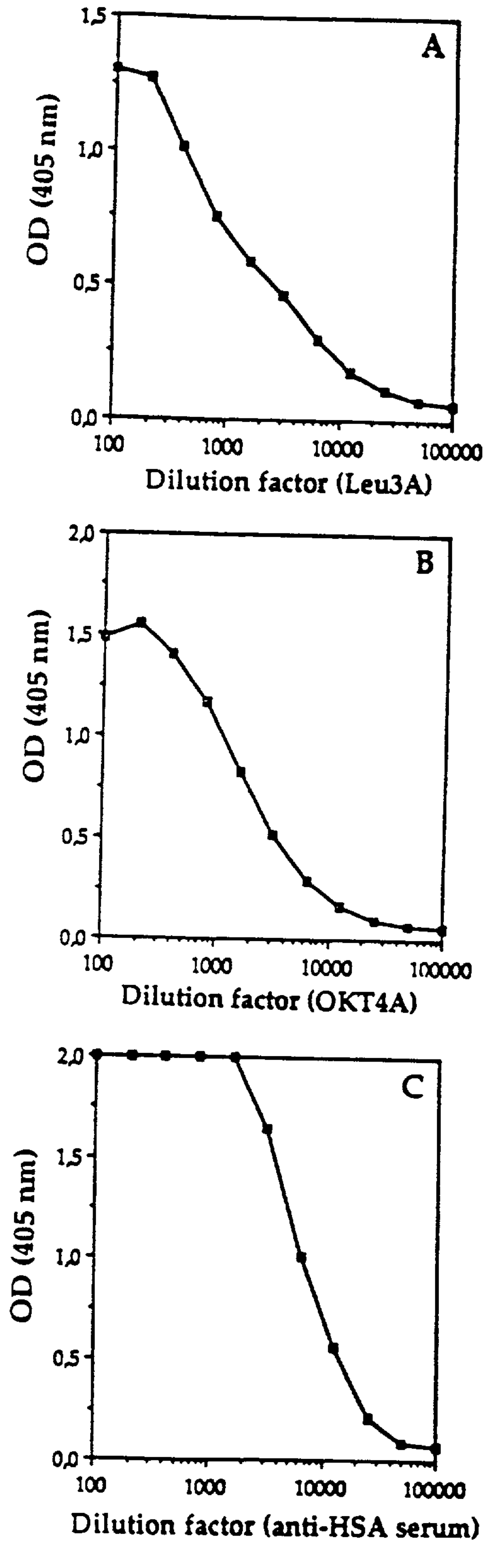

A
OD (405 nm)
1,5
1,0
0,5
0,0
100
1000
10000
100000
Dilution factor (Leu3A)
B
OD (405 nm)
2,0
1,5
1,0
0,5
0,0
100
1000
10000
100000
Dilution factor (OKT4A)
C
OD (405 nm)
2,0
1,5
1,0
0,5
0,0
100
1000
10000
100000
Dilution factor (anti-HSA serum)

Figure 19

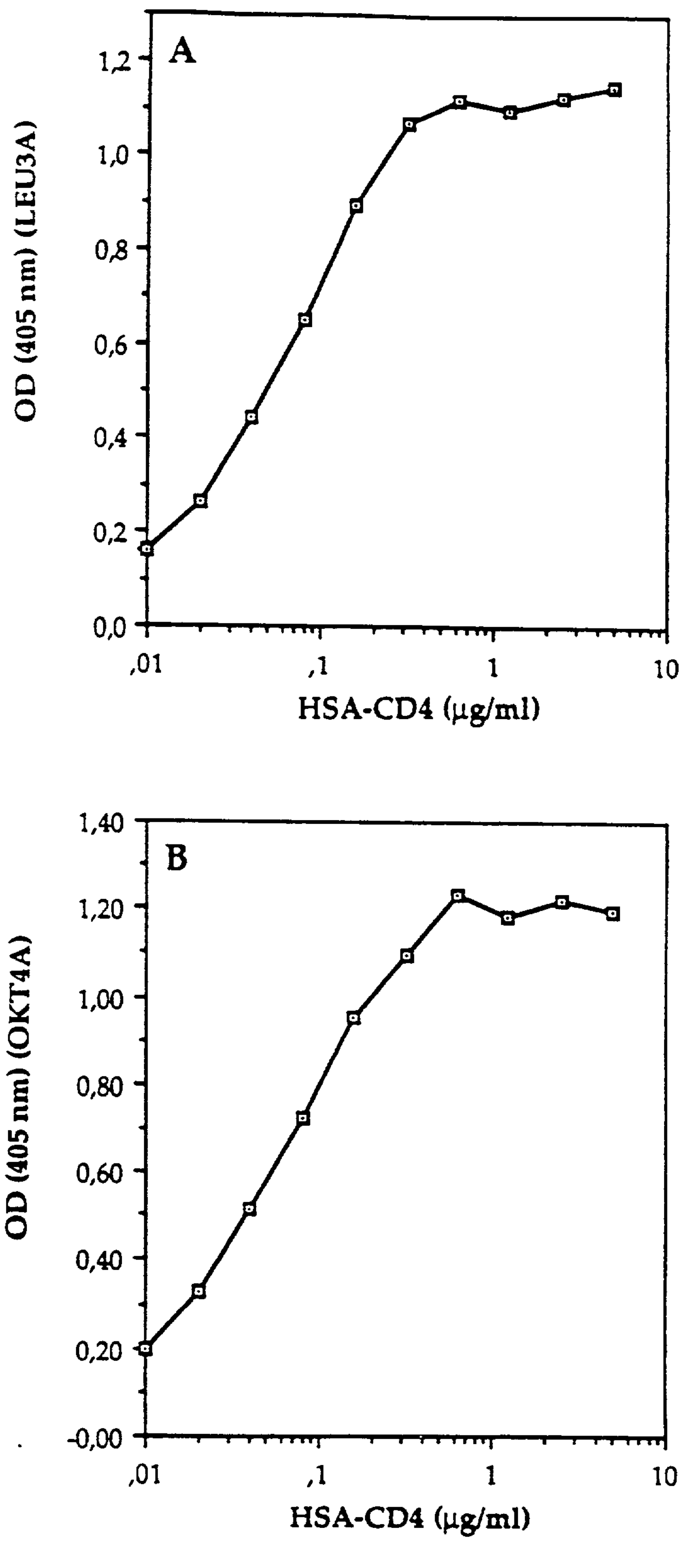

A
OD (405 nm) (LEU3A)
1,2
1,0
0,8
0,6
0,4
0,2
0,0
,01
,1
1
10
HSA-CD4 (µg/ml)
B
OD (405 nm) (OKT4A)
1,40
1,20
1,00
0,80
0,60
0,40
0,20
-0,00
,01
,1
1
10
HSA-CD4 (µg/ml)

Figure 20

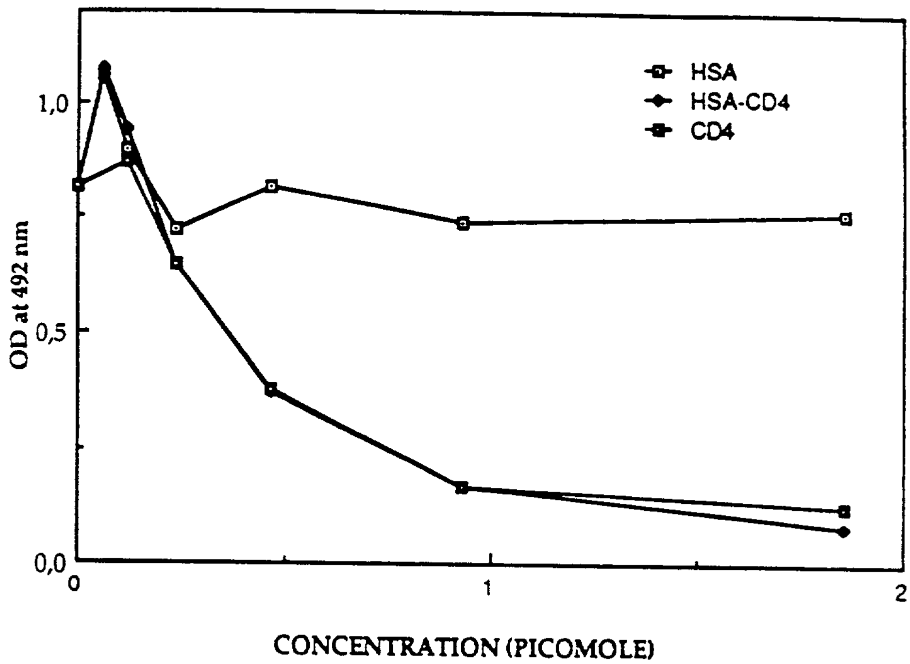

HSA
HSA-CD4
CD4
1,0
0,5
0,0
0
1
2
OD at 492 nm
CONCENTRATION (PICOMOLE)

Figure 21

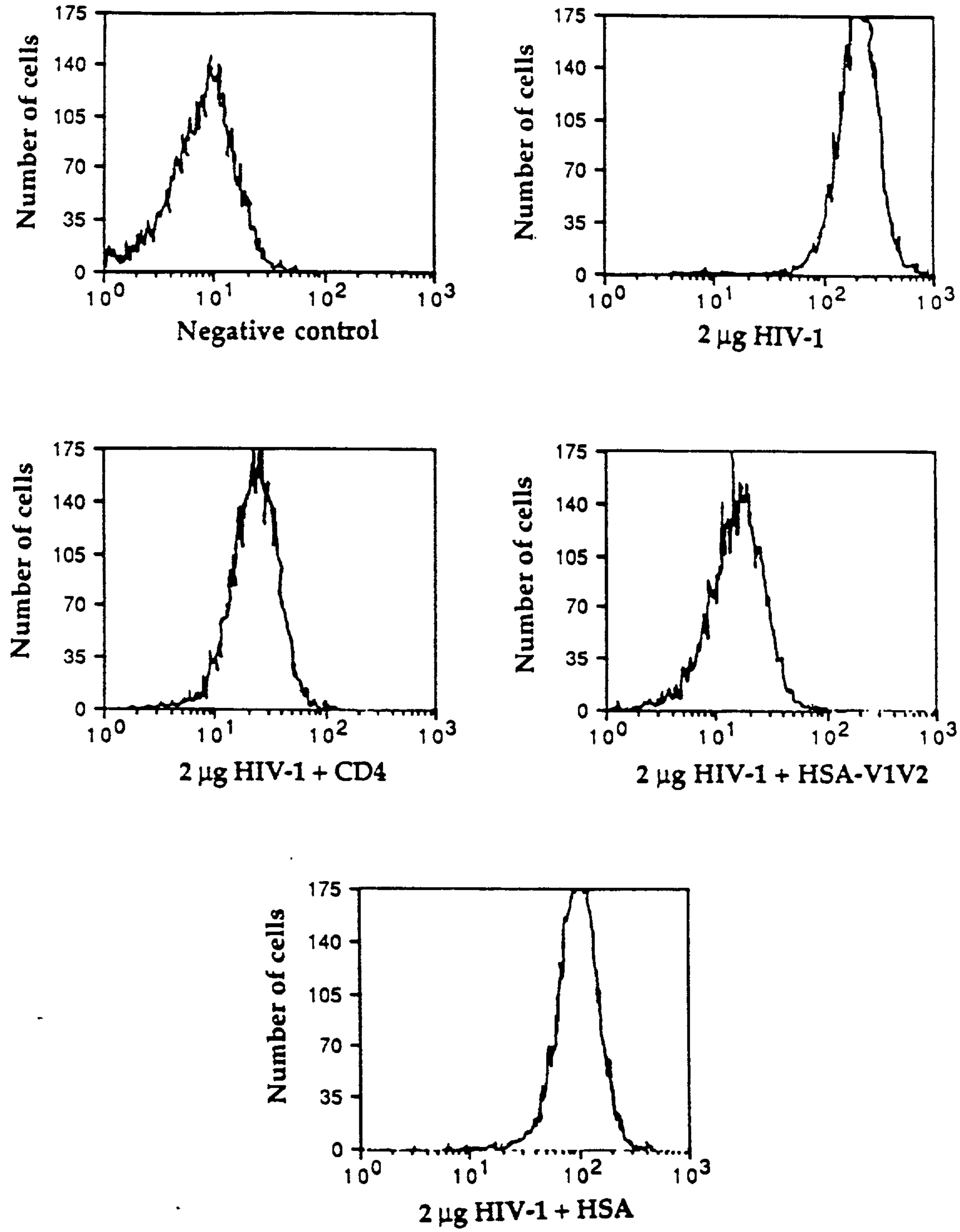

Number of cells
175
140
105
70
35
0
10^0
10^1
10^2
10^3
Negative control
2 μg HIV-1
2 μg HIV-1 + CD4
2 μg HIV-1 + HSA-V1V2
2 μg HIV-1 + HSA

Figure 22A

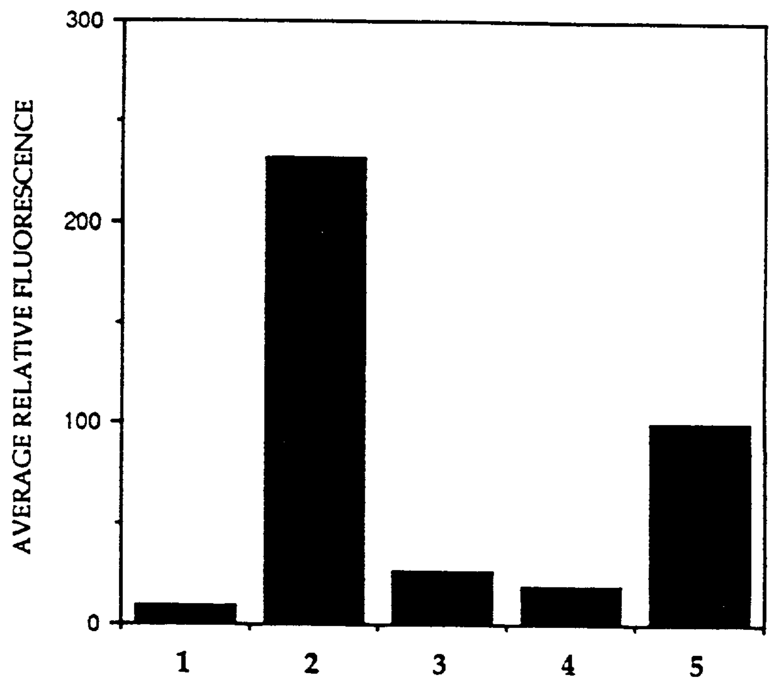
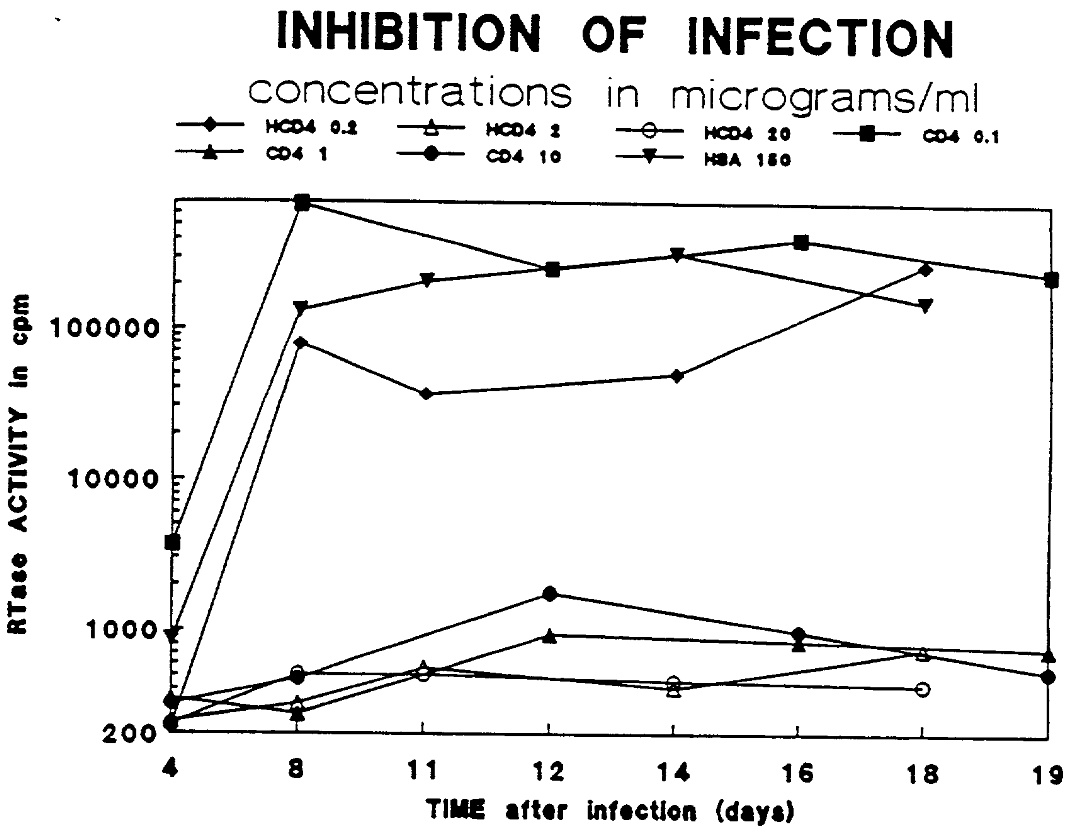


Figure 22B



INHIBITION OF INFECTION
concentrations in micrograms/ml
HCD4 0.2
HCD4 2
HCD4 20
CD4 0.1
CD4 1
CD4 10
HSA 150
RTase ACTIVITY in cpm
100000
10000
1000
200
4
8
11
12
14
16
18
19
TIME after infection (days)

Figure 23

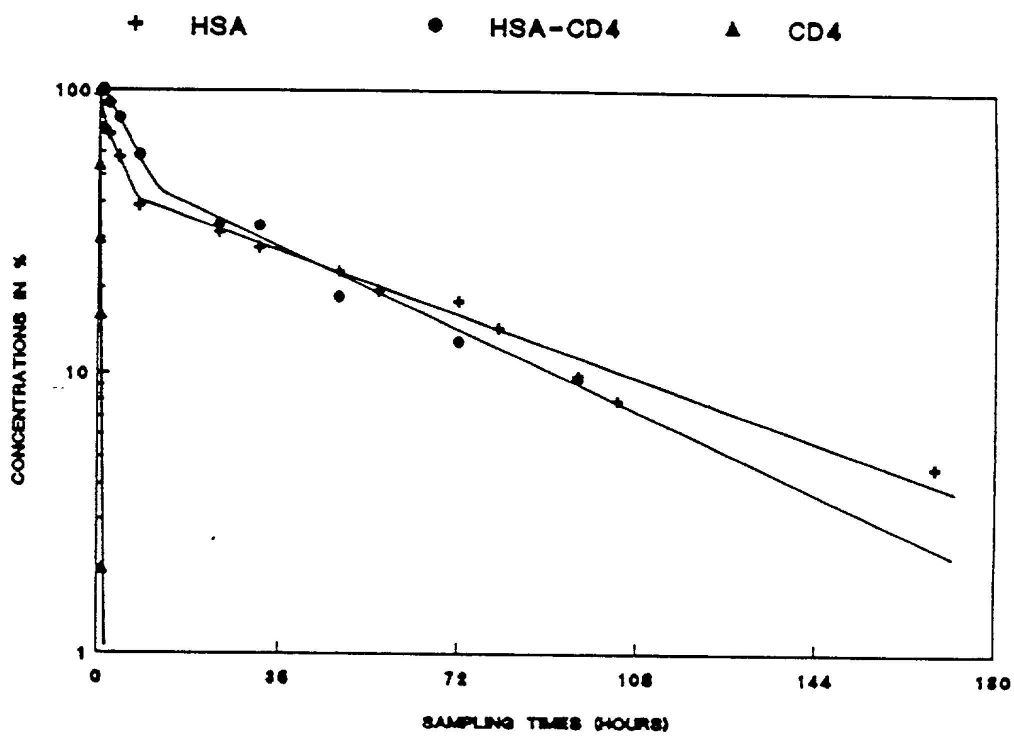

+ HSA
● HSA-CD4
▲ CD4
CONCENTRATIONS IN %
100
10
1
0
36
72
108
144
180
SAMPLING TIMES (HOURS)

Figure 24

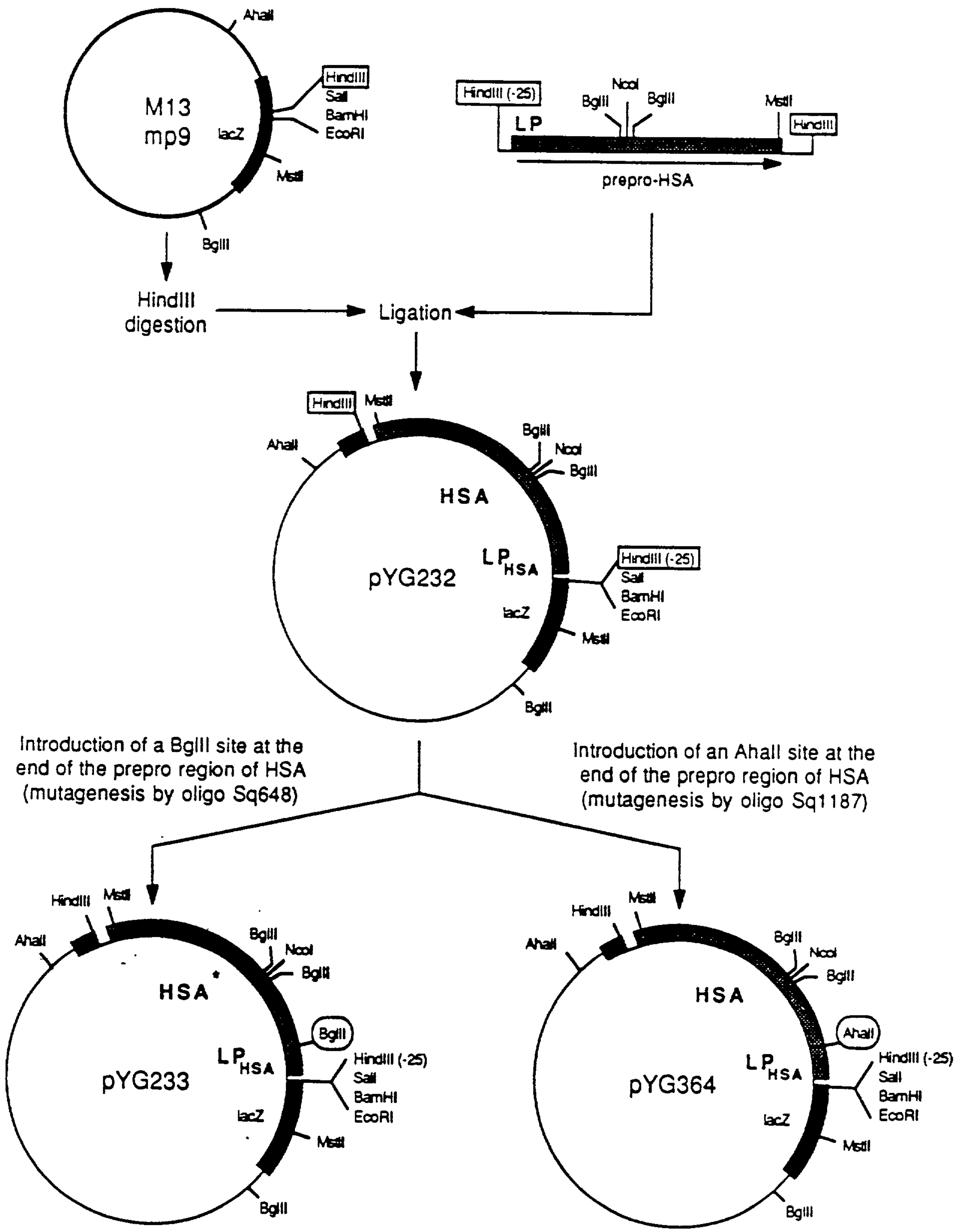

AhaII
HindIII
SalI
BamHI
EcoRI
M13
mp9
lacZ
MstII
BglII
HindIII (-25)
NcoI
BglII
BglII
MstII
HindIII
LP
prepro-HSA
HindIII
digestion
Ligation
HindIII
MstII
AhaII
BglII
NcoI
BglII
HSA
LP HSA
pYG232
HindIII (-25)
SalI
BamHI
EcoRI
lacZ
MstII
BglII
Introduction of a BglII site at the
end of the prepro region of HSA
(mutagenesis by oligo Sq648)
Introduction of an AhaII site at the
end of the prepro region of HSA
(mutagenesis by oligo Sq1187)
HindIII
MstII
AhaII
BglII
NcoI
BglII
HSA*
BglII
LP HSA
pYG233
HindIII (-25)
SalI
BamHI
EcoRI
lacZ
MstII
BglII
HindIII
MstII
AhaII
BglII
NcoI
BglII
HSA
AhaII
LP HSA
pYG364
HindIII (-25)
SalI
BamHI
EcoRI
lacZ
MstII
BglII

Figure 25

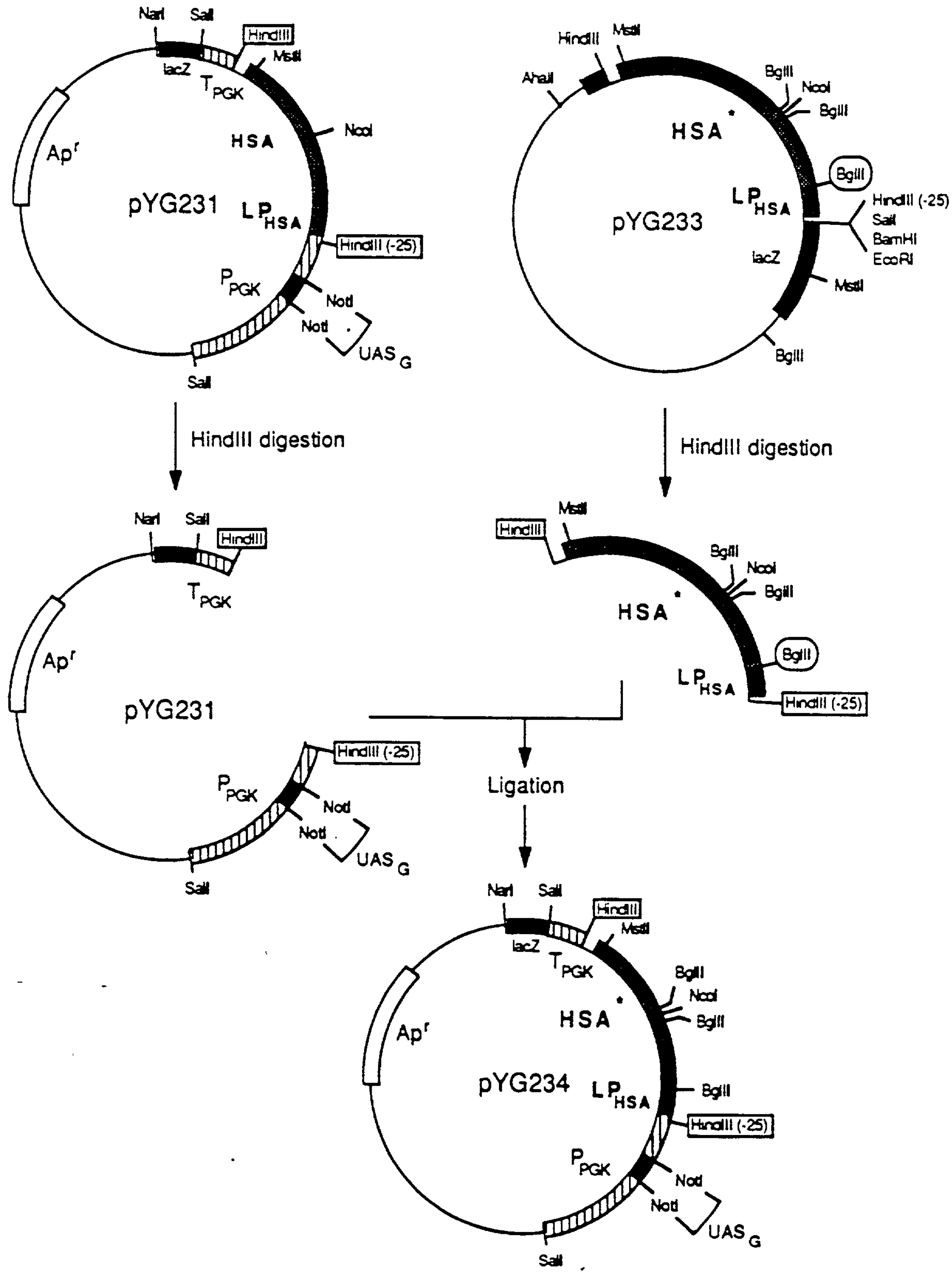

NarI
SalI
HindIII
MstII
lacZ
T_{PGK}
HSA
NcoI
Ap^r
pYG231
LP_{HSA}
HindIII (-25)
P_{PGK}
NotI
NotI
UAS_G
SalI
HindIII
MstII
AhaII
BglII
NcoI
BglII
HSA*
BglII
pYG233
LP_{HSA}
HindIII (-25)
SalI
BamHI
EcoRI
lacZ
MstII
BglII
HindIII digestion
HindIII digestion
Ligation
pYG234

Figure 26

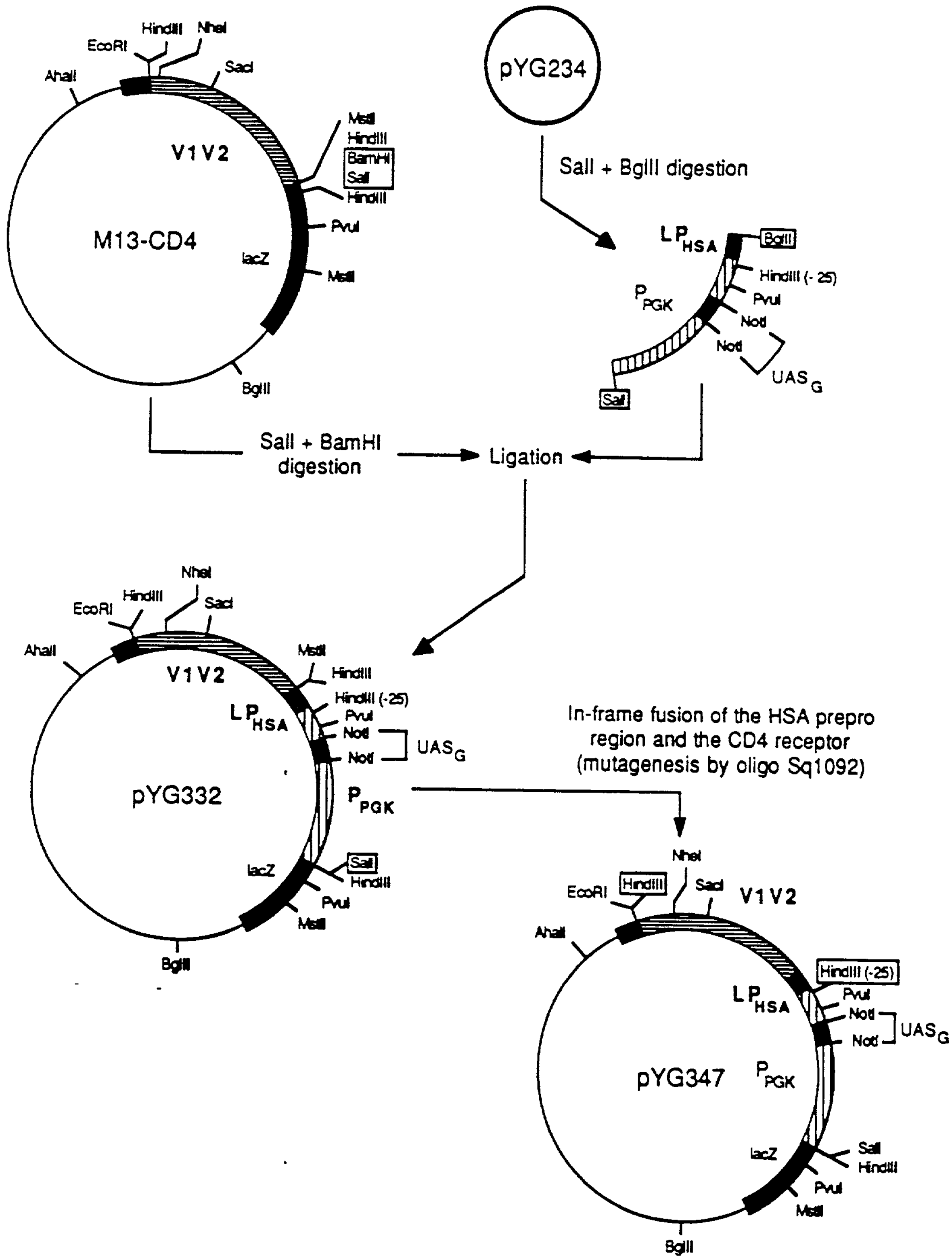

M13-CD4
V1V2
lacZ
AhaII
EcoRI
HindIII
NheI
SacI
MstII
HindIII
BamHI
SalI
HindIII
PvuI
MstII
BglII
pYG234
SalI + BglII digestion
LP HSA
BglII
HindIII (-25)
PvuI
NotI
NotI
UAS G
P PGK
SalI
SalI + BamHI digestion
Ligation
pYG332
AhaII
EcoRI
HindIII
NheI
SacI
V1V2
MstII
HindIII
HindIII (-25)
PvuI
NotI
NotI
UAS G
LP HSA
P PGK
lacZ
SalI
HindIII
PvuI
MstII
BglII
In-frame fusion of the HSA prepro region and the CD4 receptor (mutagenesis by oligo Sq1092)
pYG347
NheI
EcoRI
HindIII
SacI
V1V2
AhaII
HindIII (-25)
LP HSA
PvuI
NotI
NotI
UAS G
P PGK
lacZ
SalI
HindIII
PvuI
MstII
BglII

Figure 27

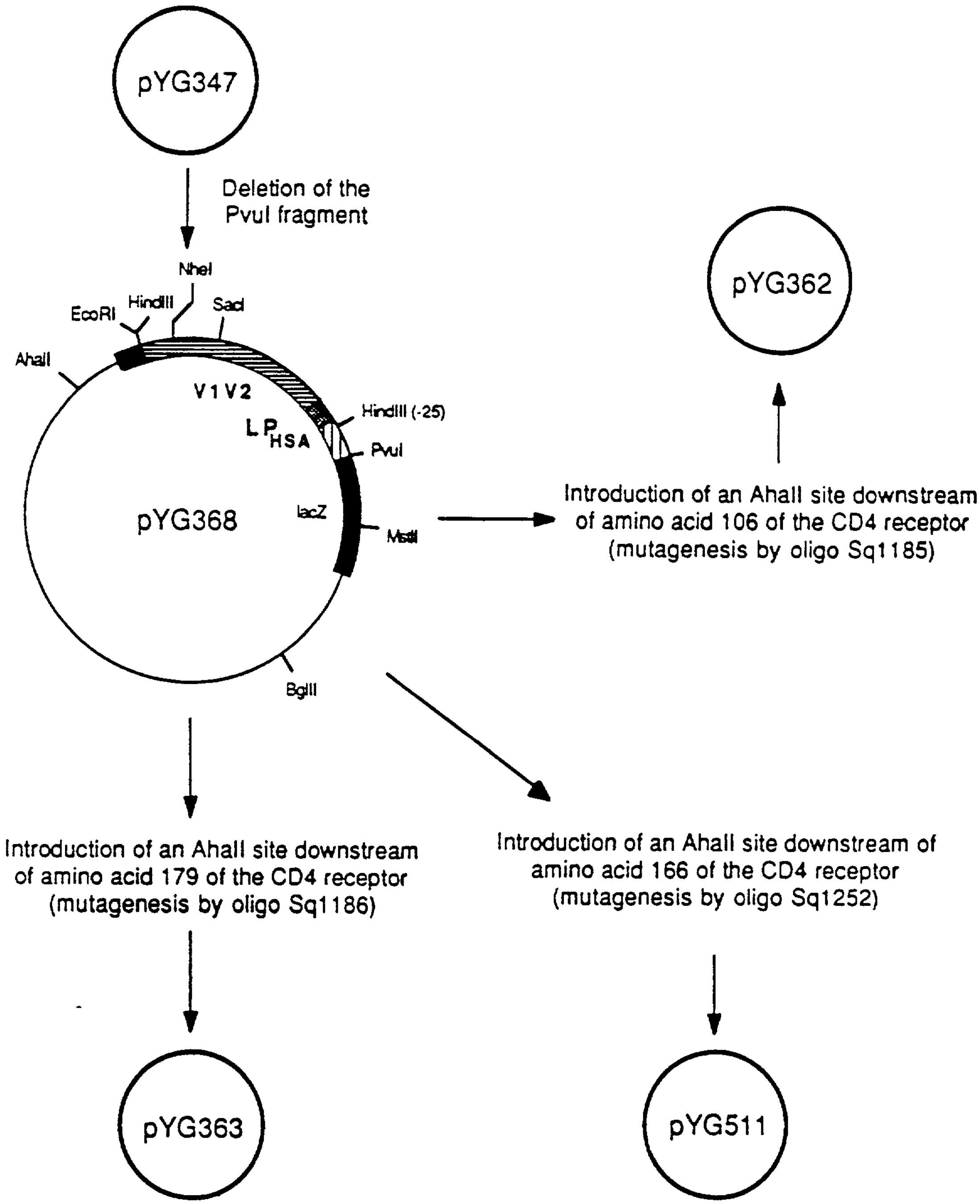

pYG347
Deletion of the
PvuI fragment
NheI
EcoRI
HindIII
SacI
AhaII
V1 V2
LP HSA
HindIII (-25)
PvuI
pYG368
lacZ
MstII
BglII
pYG362
Introduction of an AhaII site downstream
of amino acid 106 of the CD4 receptor
(mutagenesis by oligo Sq1185)
Introduction of an AhaII site downstream
of amino acid 179 of the CD4 receptor
(mutagenesis by oligo Sq1186)
Introduction of an AhaII site downstream of
amino acid 166 of the CD4 receptor
(mutagenesis by oligo Sq1252)
pYG363
pYG511

Figure 28

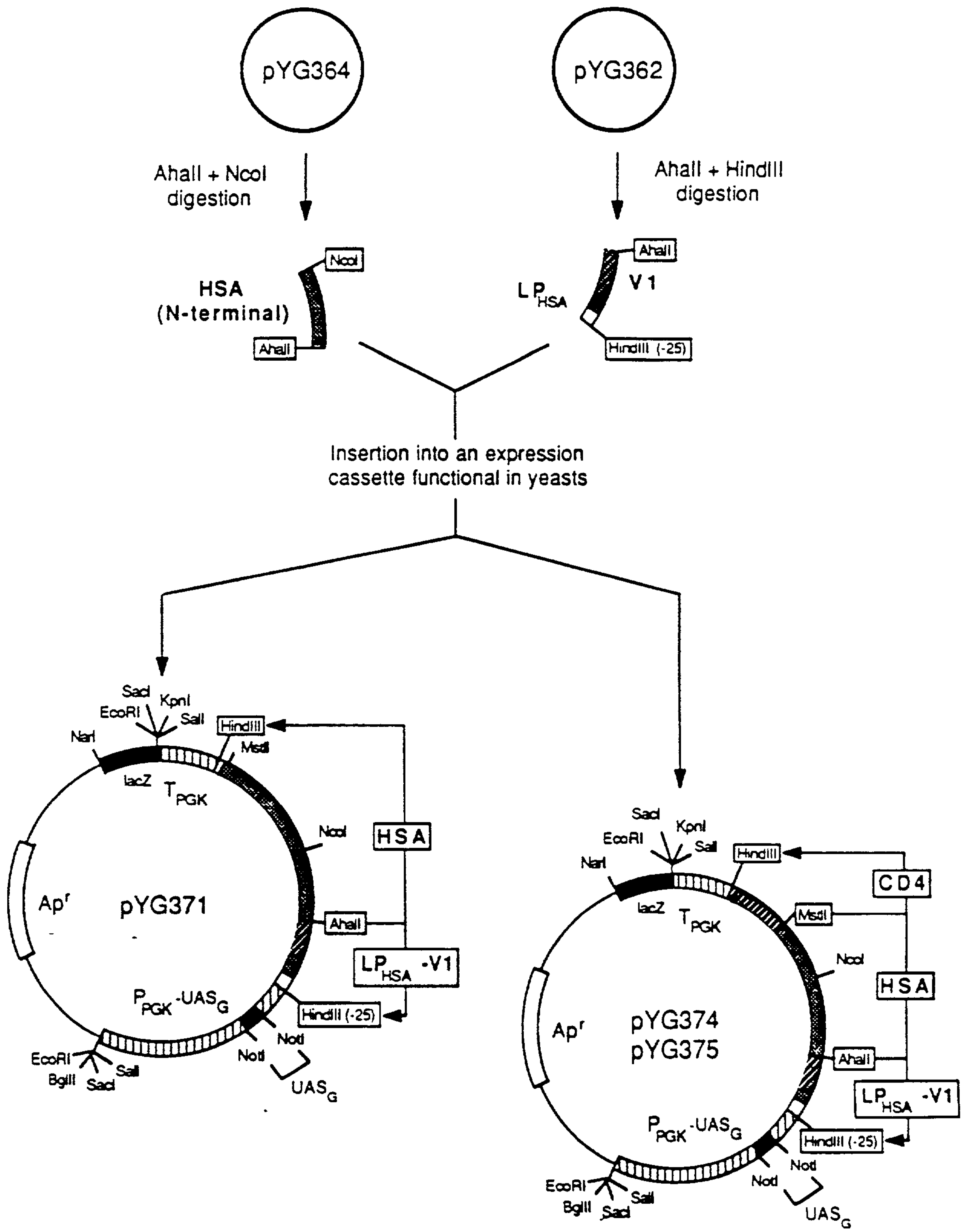

pYG364
pYG362
AhaII + NcoI
digestion
AhaII + HindIII
digestion
NcoI
HSA
(N-terminal)
AhaII
AhaII
LP HSA
V1
HindIII (-25)
Insertion into an expression
cassette functional in yeasts
SacI
KpnI
EcoRI
SalI
NarI
HindIII
MstII
lacZ
T PGK
NcoI
HSA
Ap r
pYG371
AhaII
LP HSA -V1
P PGK -UAS G
HindIII (-25)
NotI
NotI
UAS G
EcoRI
SalI
BglII
SacI
CD4
pYG374
pYG375

Figure 29

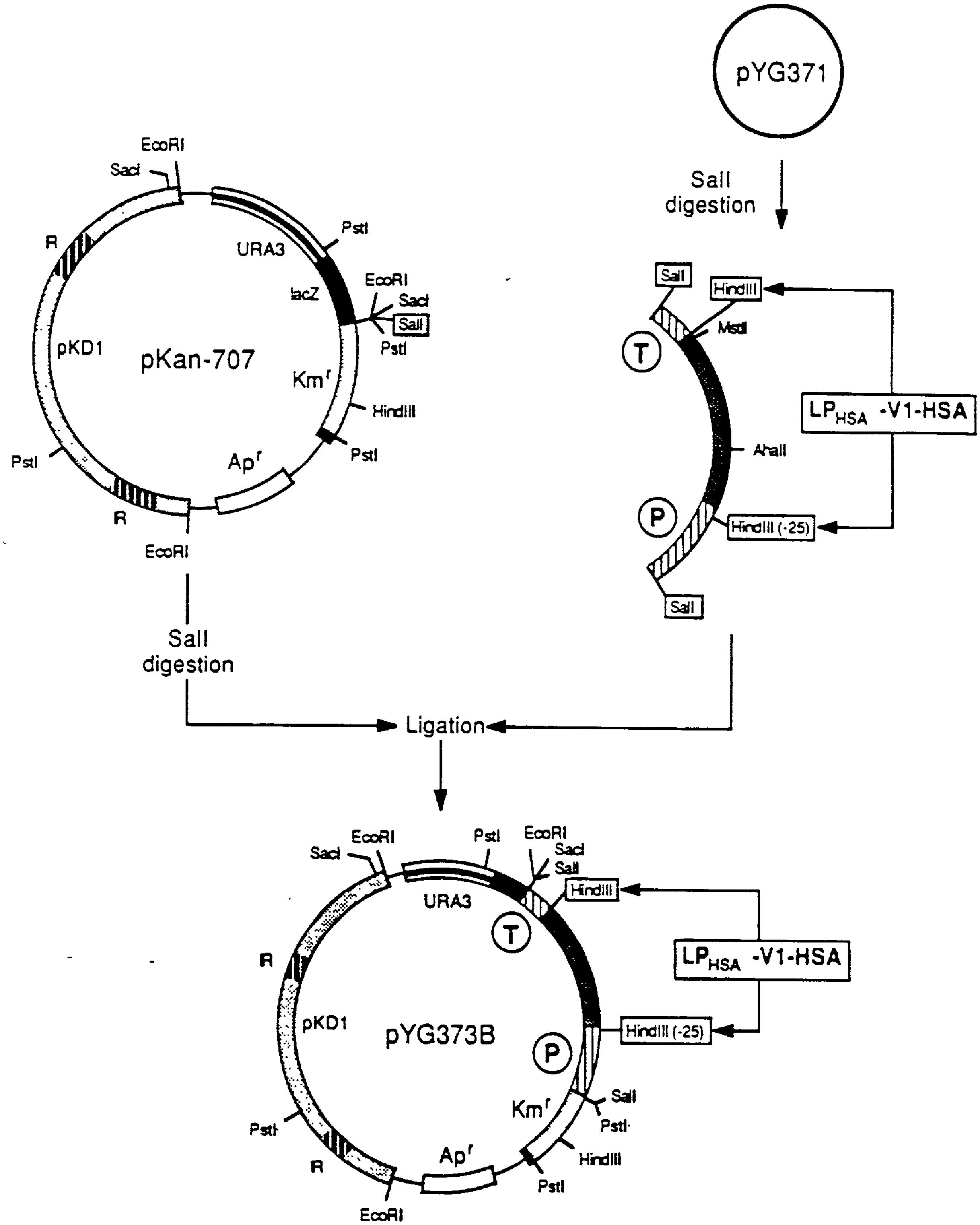

pYG371
SalI
digestion
SalI
HindIII
MstII
T
LPHSA -V1-HSA
AhaII
P
HindIII (-25)
SalI
EcoRI
SacI
R
URA3
PstI
lacZ
EcoRI
SacI
SalI
PstI
pKD1
pKan-707
Kmr
HindIII
PstI
Apr
PstI
R
EcoRI
SalI
digestion
Ligation
SacI
EcoRI
PstI
EcoRI
SacI
SalI
HindIII
URA3
T
LPHSA -V1-HSA
R
pKD1
pYG373B
HindIII (-25)
P
SalI
PstI
Kmr
HindIII
Apr
PstI
PstI
R
EcoRI

Figure 30

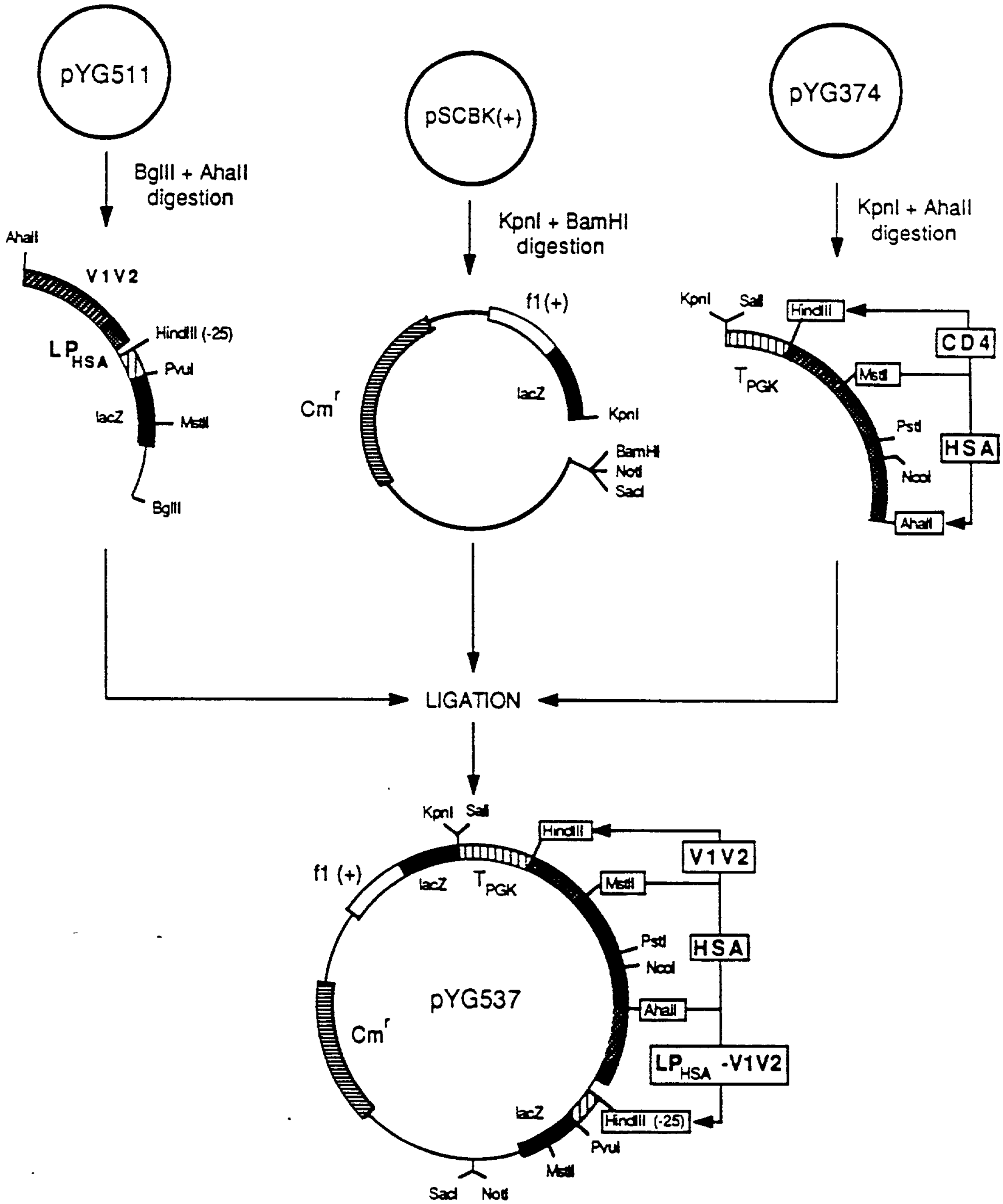
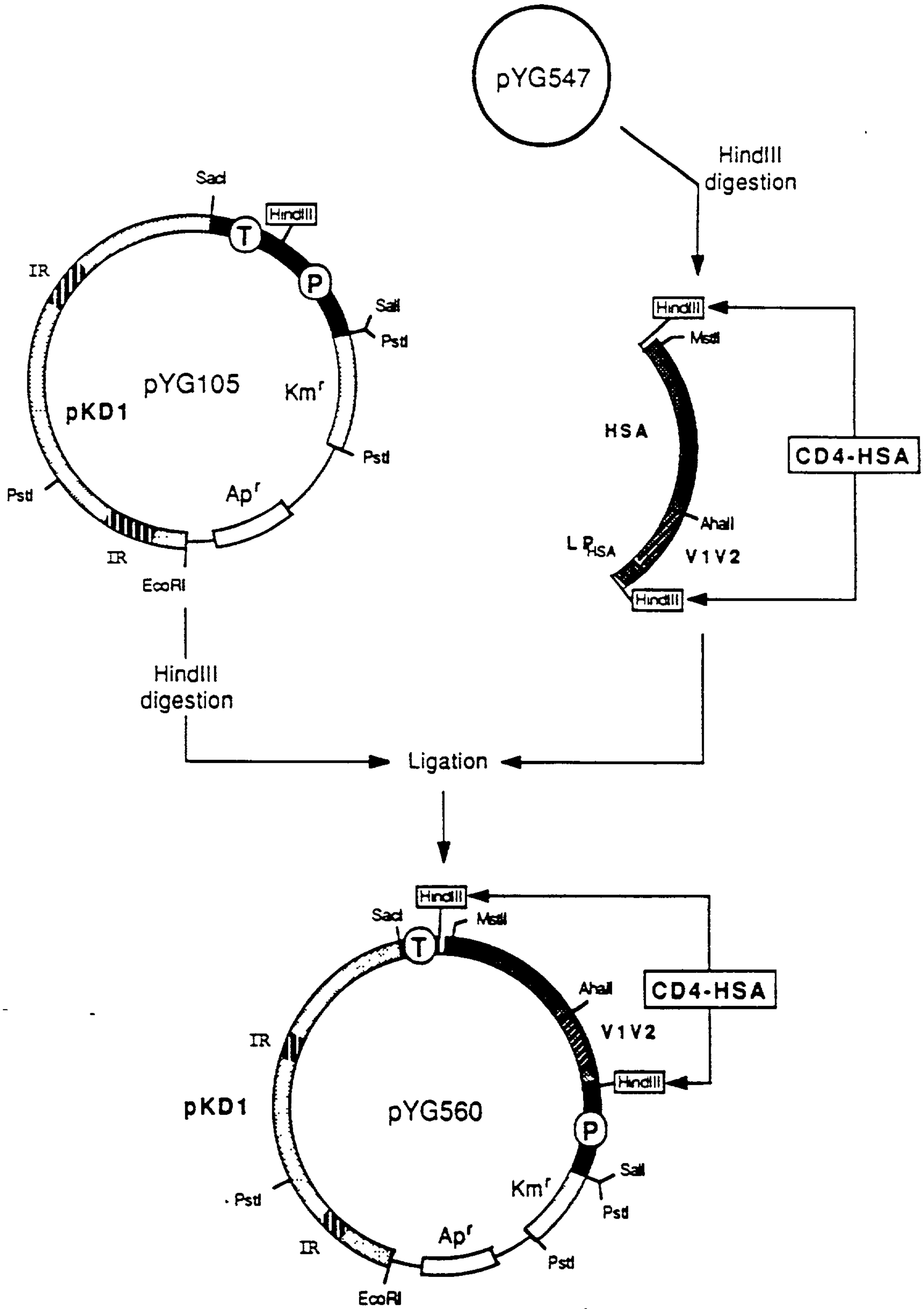

pYG511
BglII + AhaII
digestion
AhaII
V1V2
LPHSA
HindIII (-25)
PvuI
lacZ
MstII
BglII
pSCBK(+)
KpnI + BamHI
digestion
f1(+)
Cmr
lacZ
KpnI
BamHI
NotI
SacI
pYG374
KpnI + AhaII
digestion
KpnI
SalI
HindIII
CD4
TPGK
MstII
PstI
HSA
NcoI
AhaII
LIGATION
KpnI
SalI
HindIII
V1V2
f1 (+)
lacZ
TPGK
MstII
PstI
HSA
NcoI
pYG537
AhaII
Cmr
LPHSA -V1V2
lacZ
HindIII (-25)
PvuI
MstII
SacI
NotI

Figure 31



pYG547
HindIII digestion
SacI
HindIII
T
P
SalI
PstI
IR
pYG105
pKD1
Km^r
Ap^r
PstI
IR
EcoRI
HindIII
MstII
HSA
CD4-HSA
AhaII
L P$_{HSA}$
V1V2
HindIII
HindIII digestion
Ligation
HindIII
SacI
MstII
T
AhaII
CD4-HSA
IR
V1V2
HindIII
pKD1
pYG560
P
SalI
Km^r
PstI
PstI
Ap^r
IR
PstI
EcoRI

Figure 32

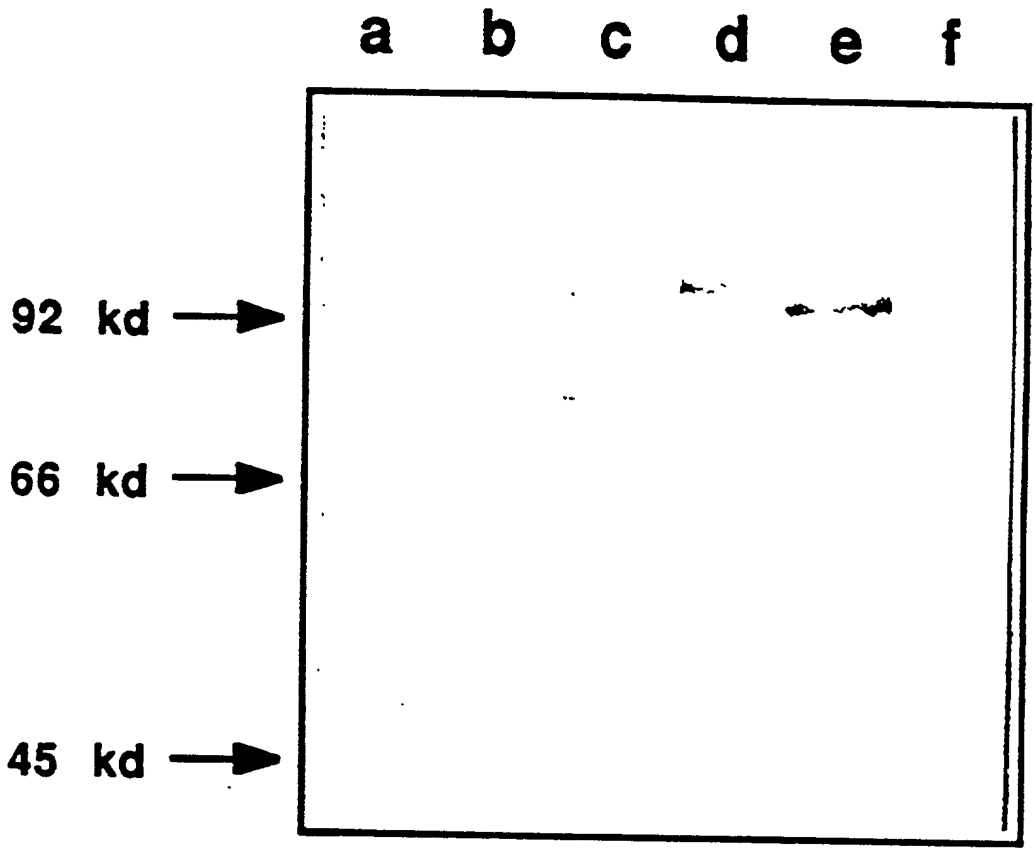


Figure 33

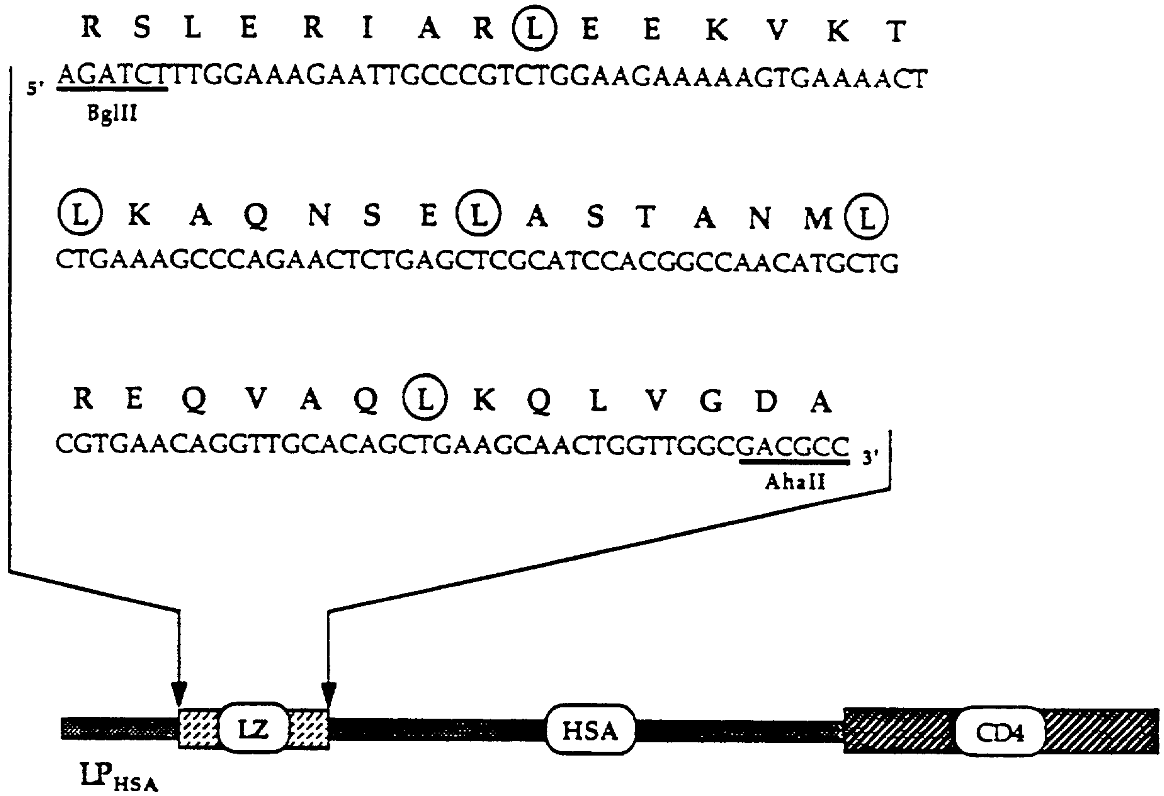

R S L E R I A R L E E K V K T
5' AGATCTTTGGAAAGAATTGCCCGTCTGGAAGAAAAAGTGAAAACT
BglII
L K A Q N S E L A S T A N M L
CTGAAAGCCCAGAACTCTGAGCTCGCATCCACGGCCAACATGCTG
R E Q V A Q L K Q L V G D A
CGTGAACAGGTTGCACAGCTGAAGCAACTGGTTGGCGACGCC 3'
AhaII
LZ
HSA
CD4
LP HSA

Figure 34

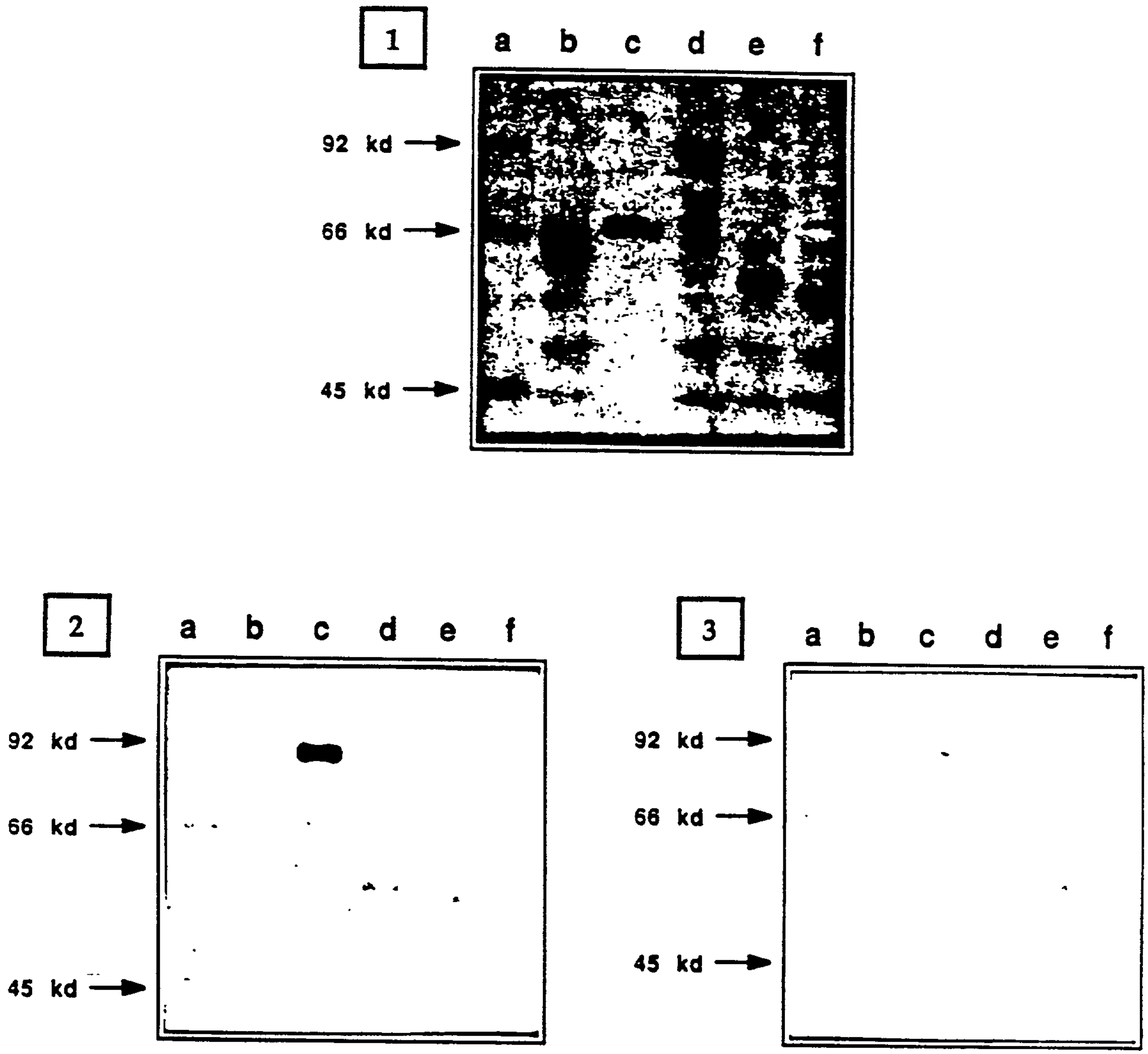

1
a b c d e f
92 kd
66 kd
45 kd
2
a b c d e f
92 kd
66 kd
45 kd
3
a b c d e f
92 kd
66 kd
45 kd

Figure 35

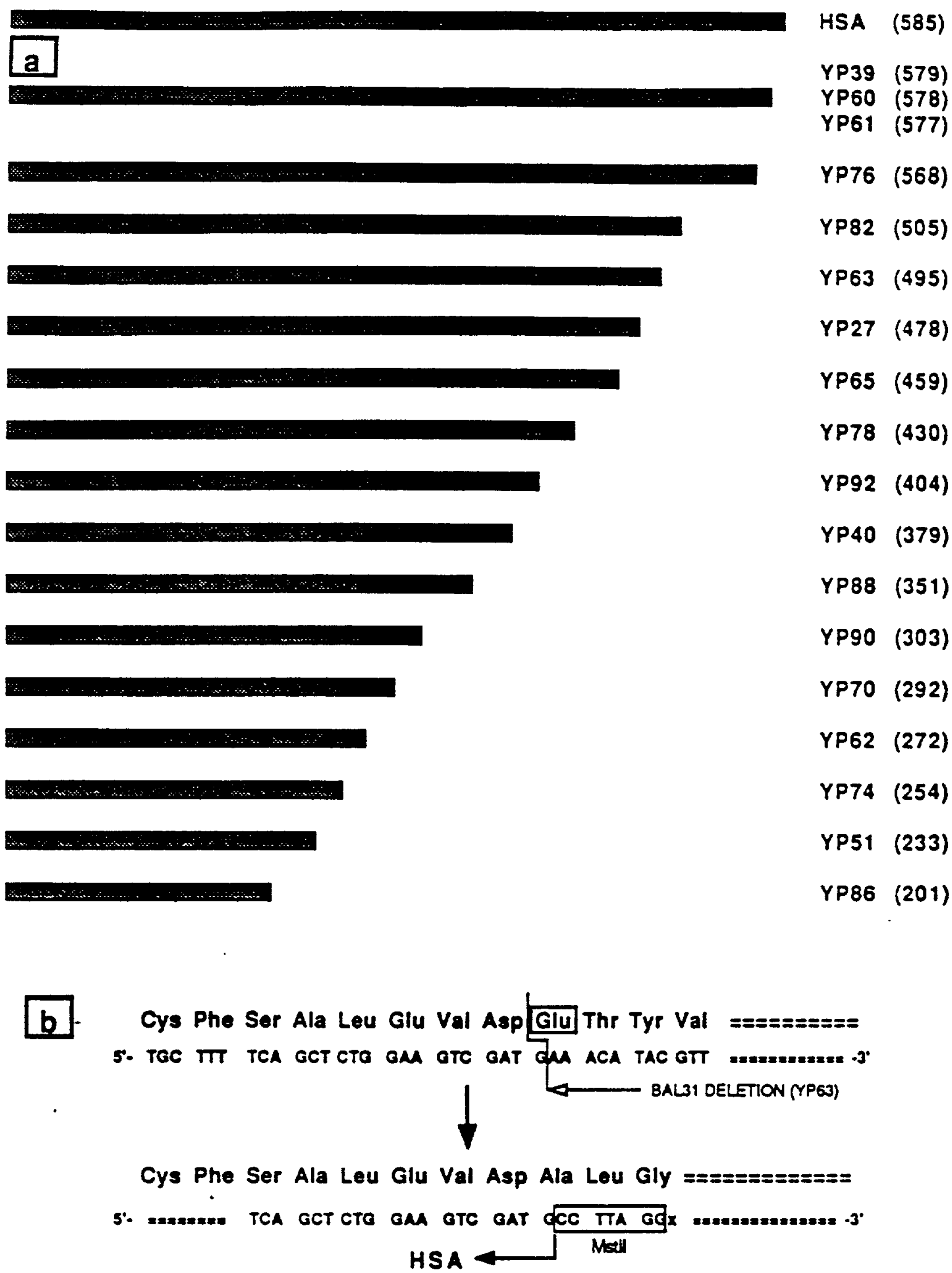

a
HSA (585)
YP39 (579)
YP60 (578)
YP61 (577)
YP76 (568)
YP82 (505)
YP63 (495)
YP27 (478)
YP65 (459)
YP78 (430)
YP92 (404)
YP40 (379)
YP88 (351)
YP90 (303)
YP70 (292)
YP62 (272)
YP74 (254)
YP51 (233)
YP86 (201)
b
Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val ==========
5'- TGC TTT TCA GCT CTG GAA GTC GAT GAA ACA TAC GTT ============ -3'
BAL31 DELETION (YP63)
Cys Phe Ser Ala Leu Glu Val Asp Ala Leu Gly ============
5'- ======== TCA GCT CTG GAA GTC GAT GCC TTA GGx =============== -3'
MstII
HSA

Figure 36

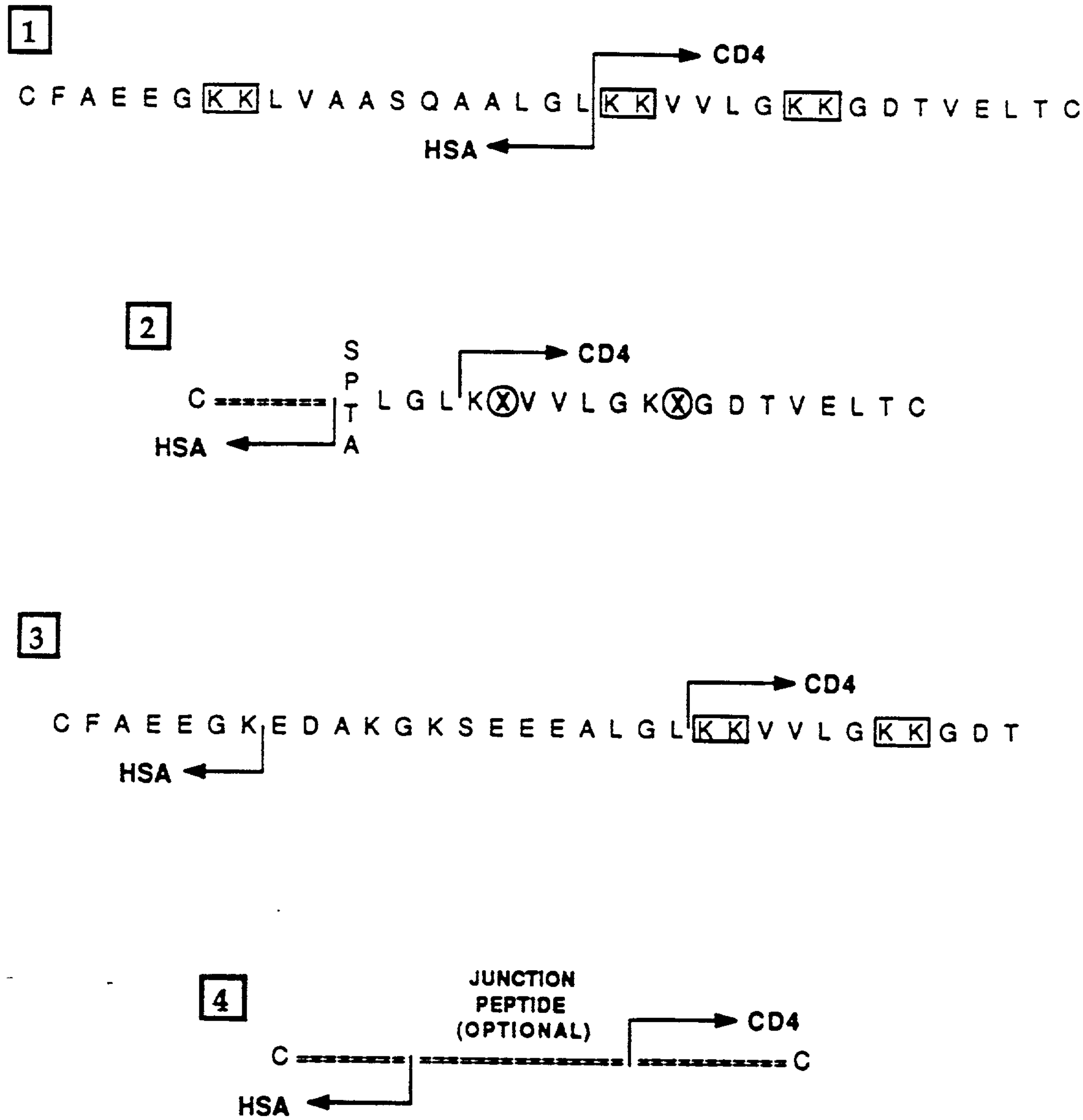

1
CD4
C F A E E G K K L V A A S Q A A L G L K K V V L G K K G D T V E L T C
HSA
2
S
P
T
A
CD4
C
L G L K X V V L G K X G D T V E L T C
HSA
3
CD4
C F A E E G K E D A K G K S E E E A L G L K K V V L G K K G D T
HSA
4
JUNCTION
PEPTIDE
(OPTIONAL)
CD4
C
C
HSA

Figure 37

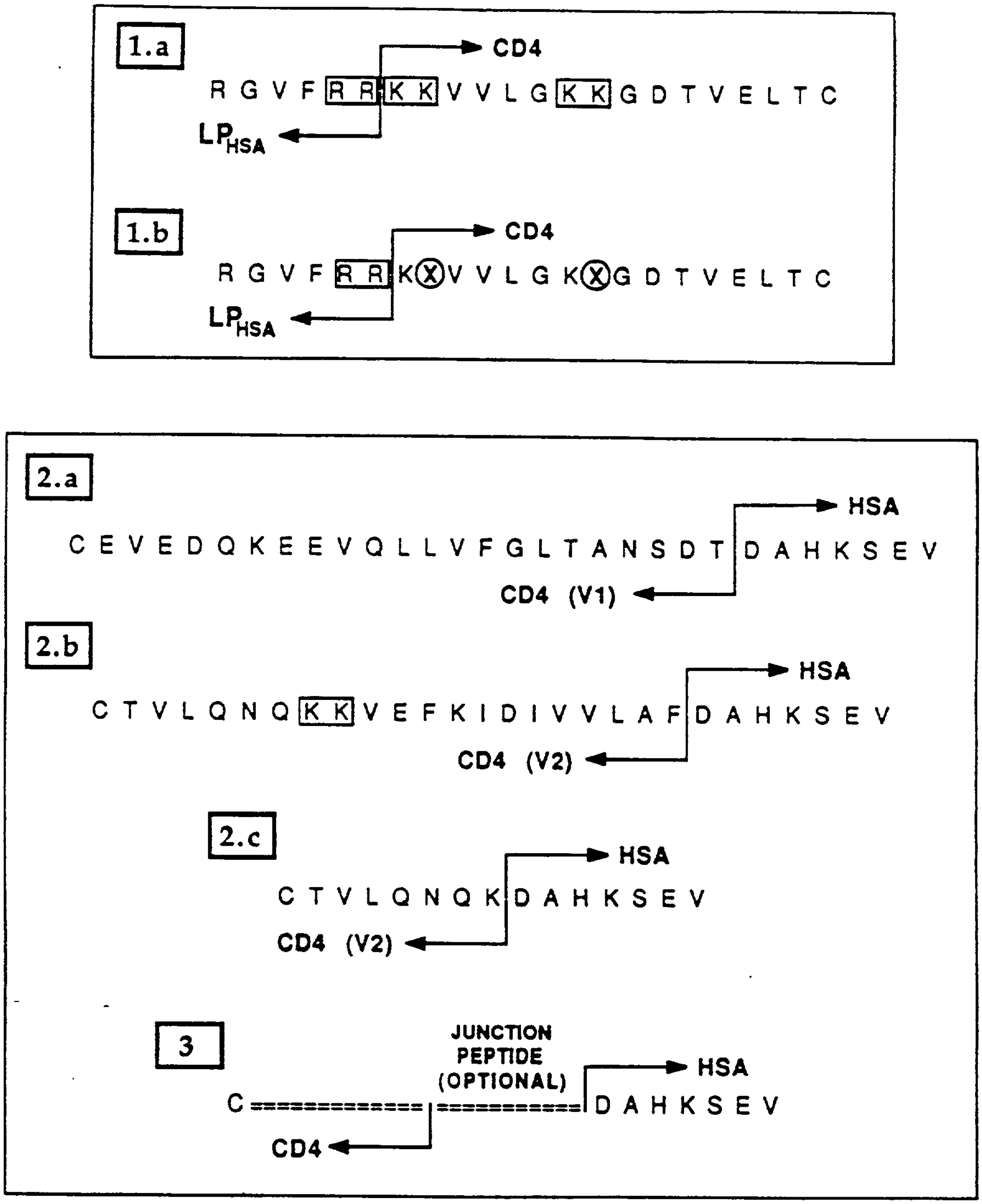

1.a
CD4
R G V F R R K K V V L G K K G D T V E L T C
LP HSA
1.b
CD4
R G V F R R K X V V L G K X G D T V E L T C
LP HSA
2.a
HSA
C E V E D Q K E E V Q L L V F G L T A N S D T D A H K S E V
CD4 (V1)
2.b
HSA
C T V L Q N Q K K V E F K I D I V V L A F D A H K S E V
CD4 (V2)
2.c
HSA
C T V L Q N Q K D A H K S E V
CD4 (V2)
3
JUNCTION
PEPTIDE
(OPTIONAL)
HSA
C D A H K S E V
CD4

Figure 38